

FIG.—3

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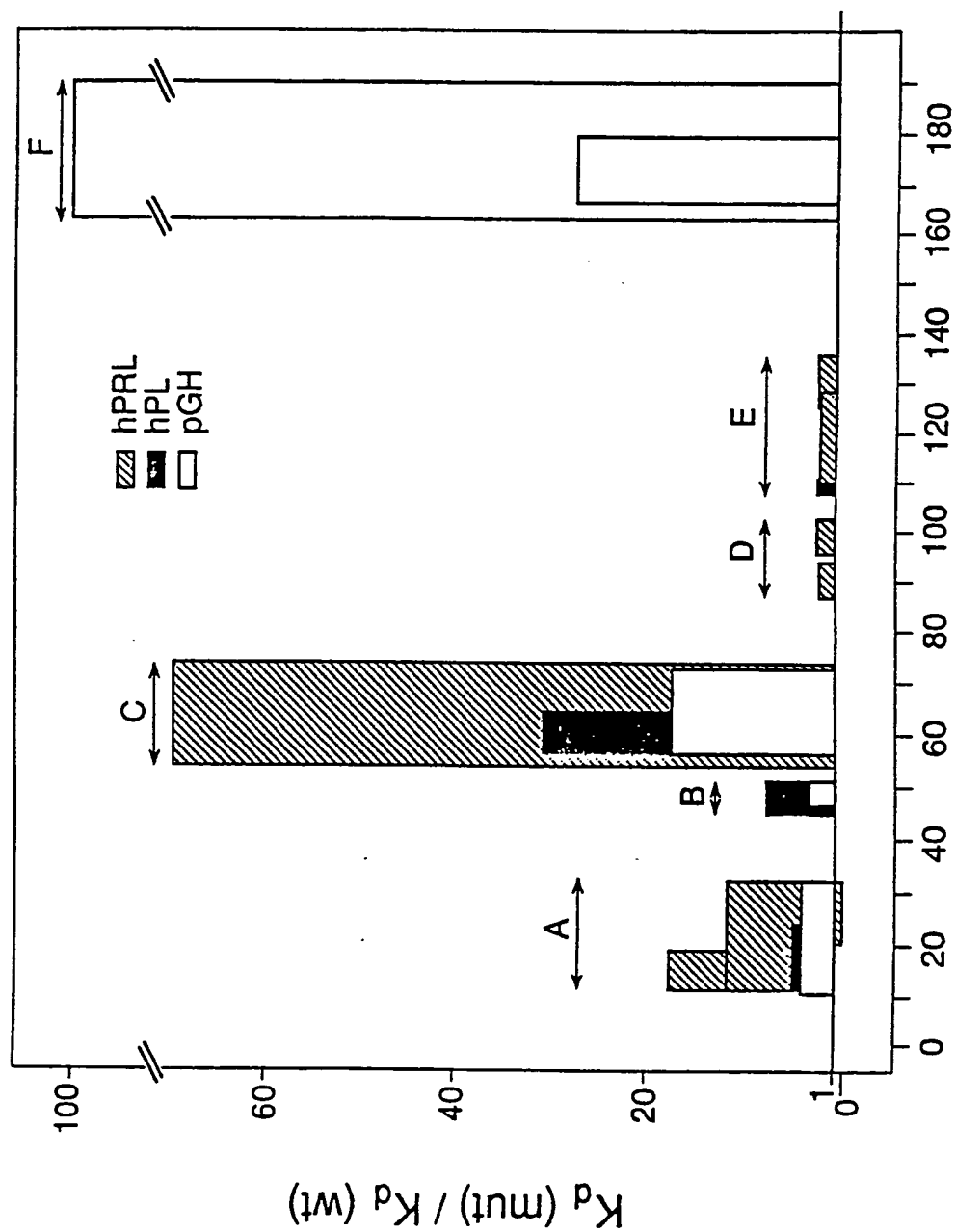


FIG.—4

Residue number in hGH

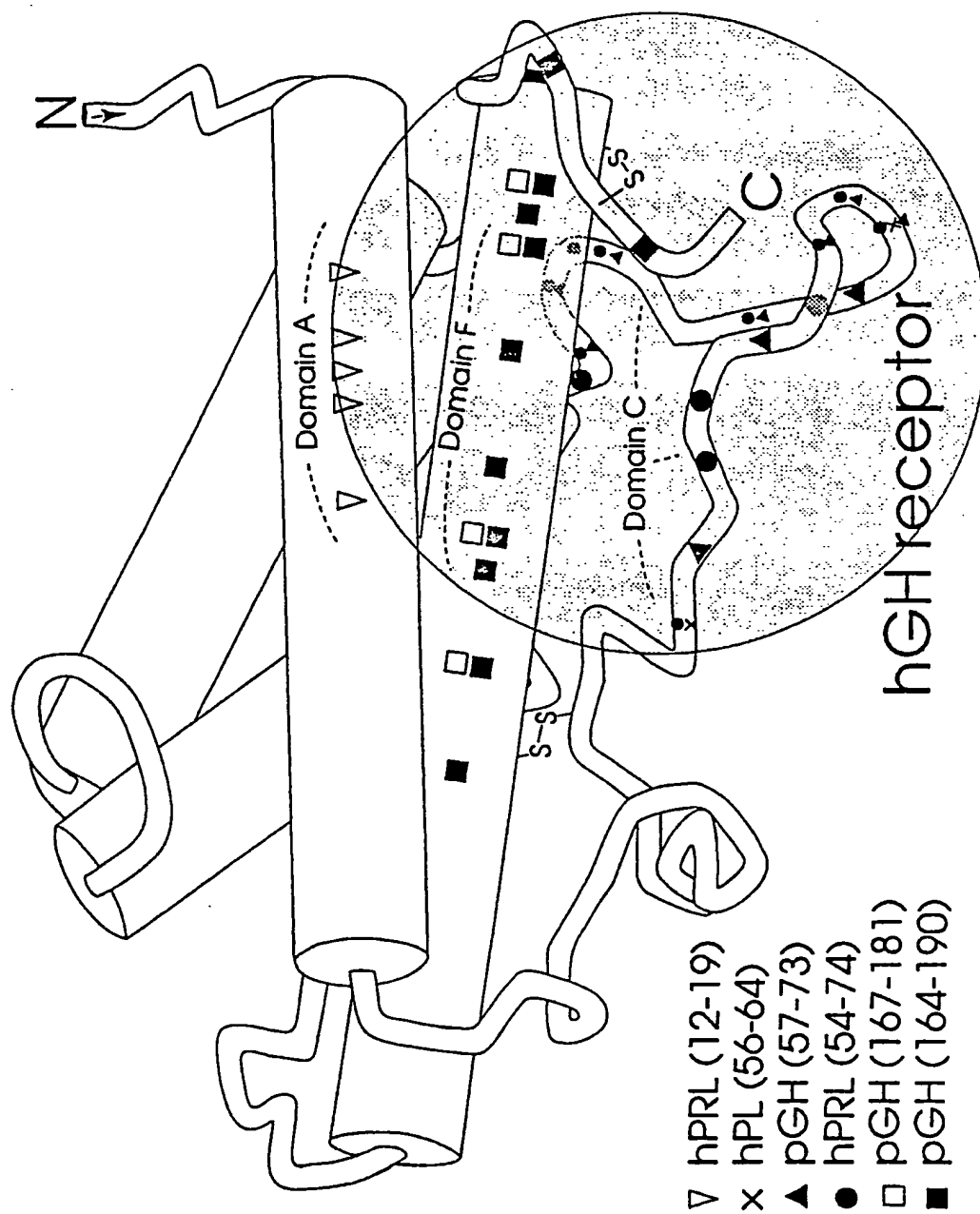


FIG.—5

- hPRL (88-95)
- ▲ hPRL (97-104)
- hPL (109-112)
- x hPRL (111-129) minus  
hPRL (126-136)

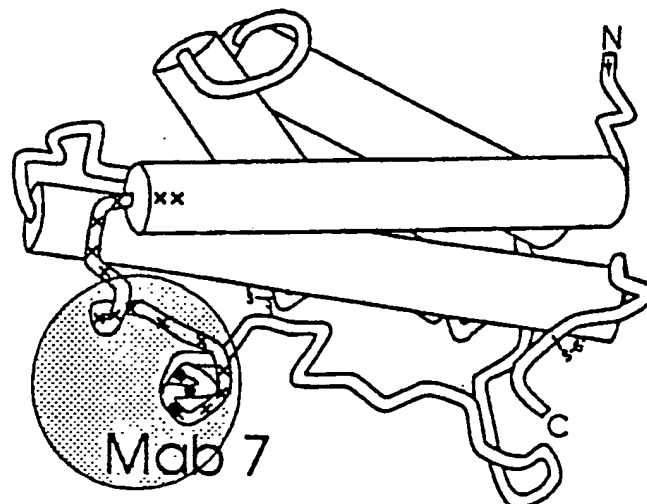
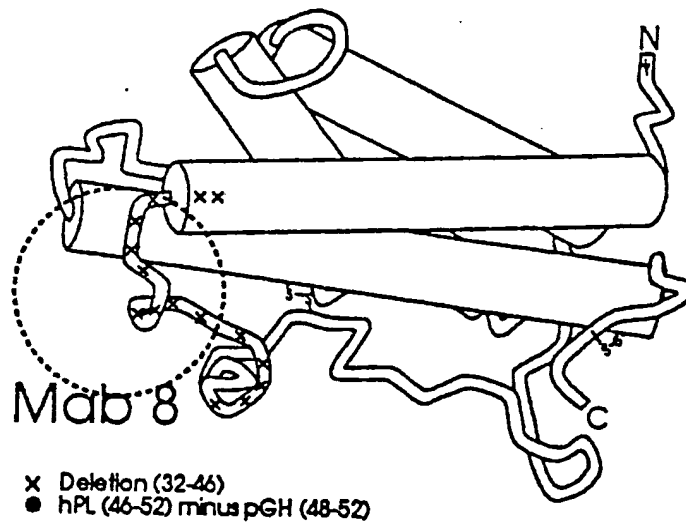
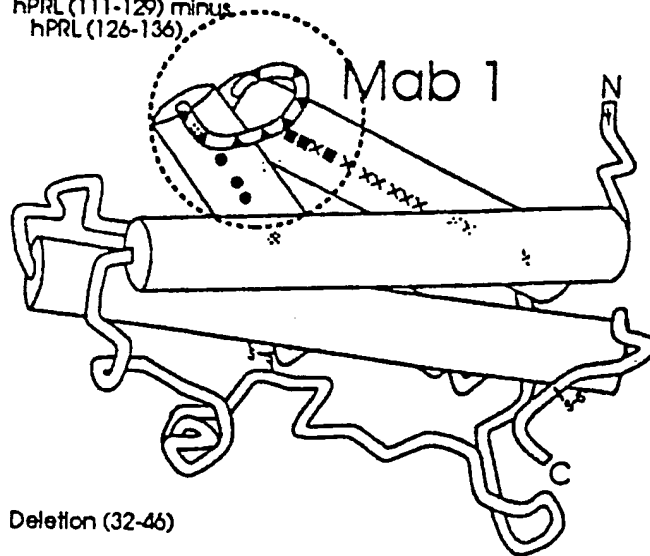
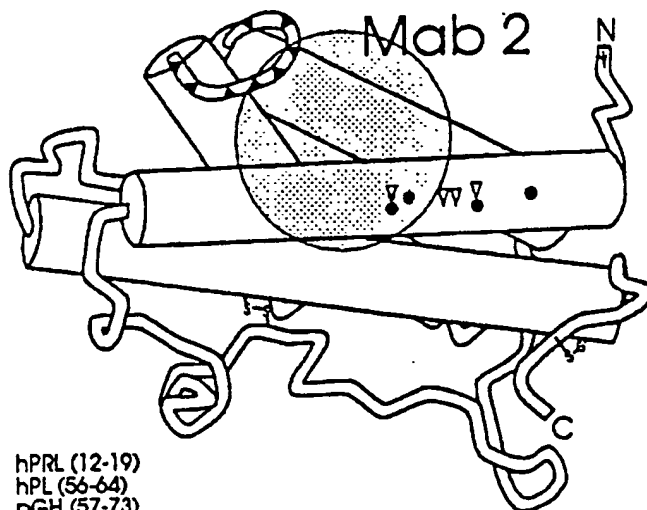


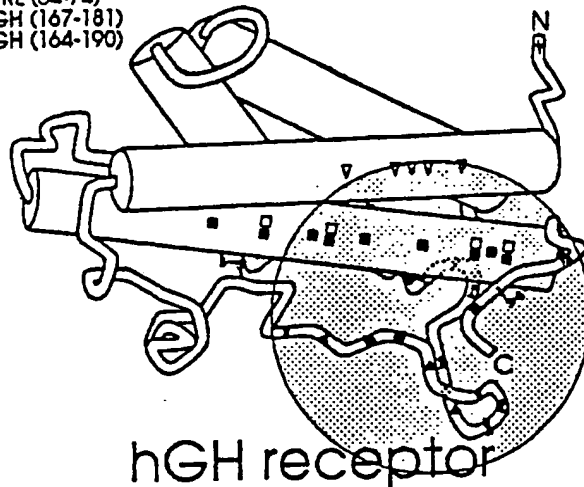
FIG.—6A

SUBSTITUTE SHEET

- pGH (11-33) minus hPRL (22-33)
- ▽ hPRL (12-19) minus hPL (12-25)
- ▲ hPRL (97-104)

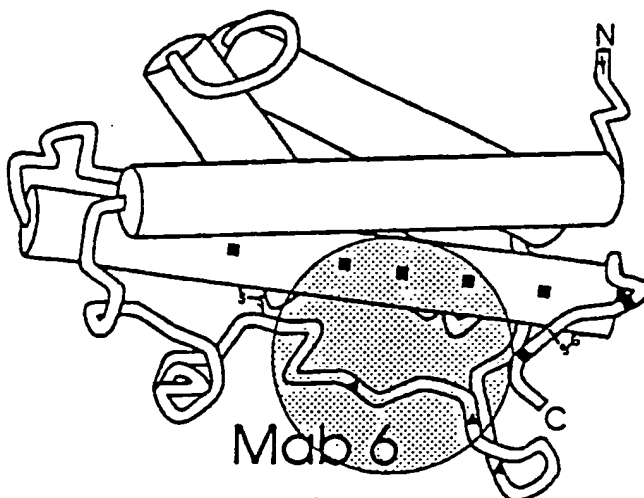


- ▽ hPRL (12-19)
- × hPL (56-64)
- ▲ pGH (57-73)
- hPRL (54-74)
- pGH (167-181)
- pGH (164-190)



hGH receptor

- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)

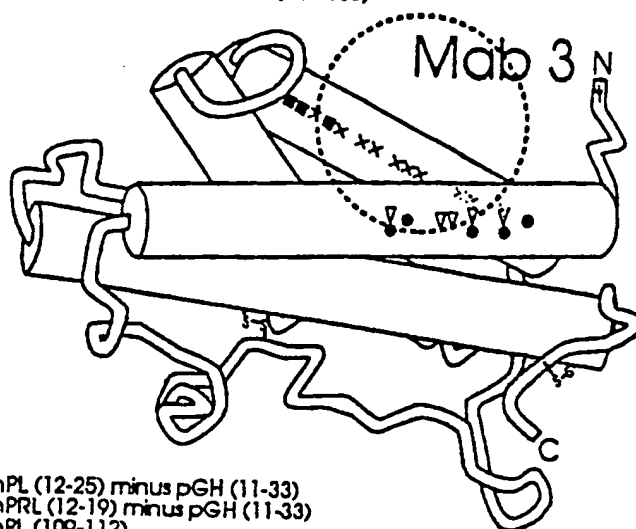


Mab 6

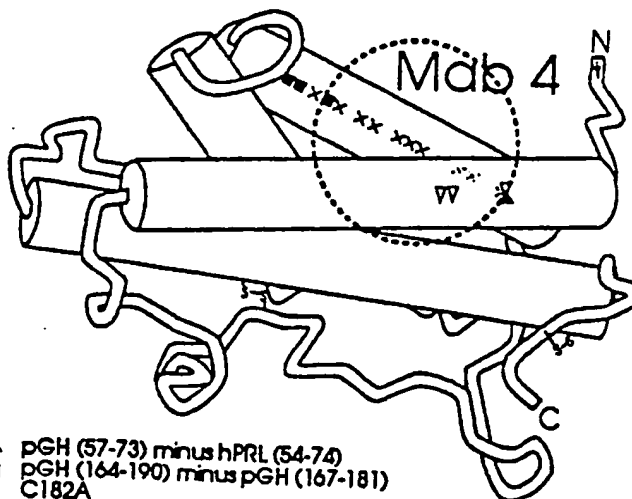
FIG.—6B

SUBSTITUTE SHEET

- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)
- C182A

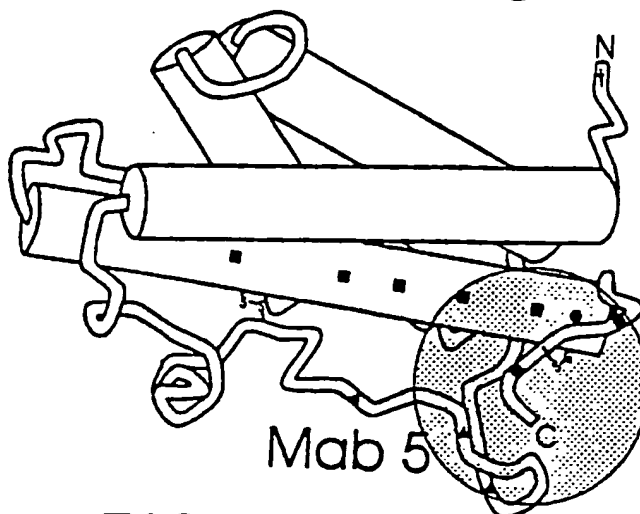


FIG.—6C

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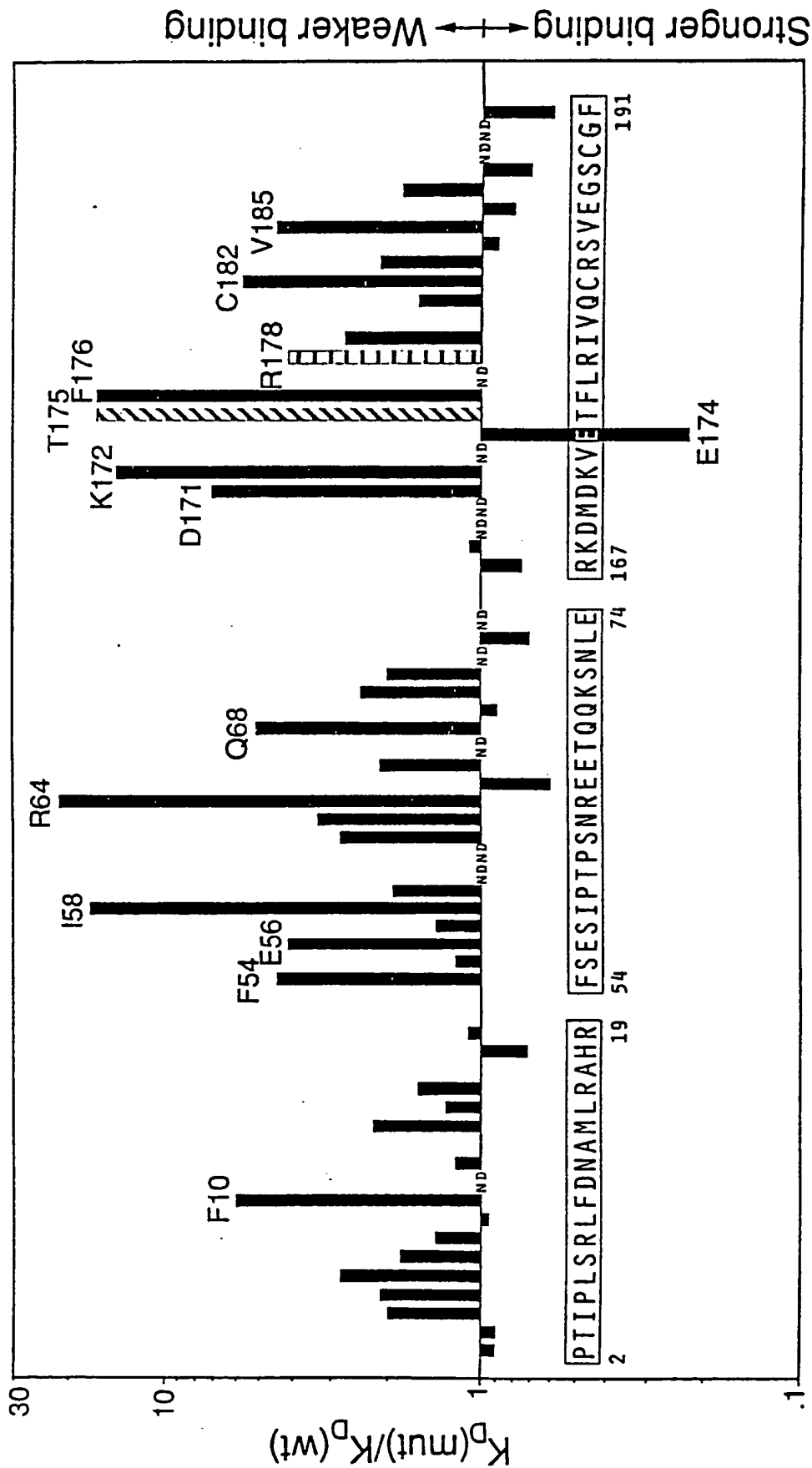


FIG.—7



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## hGH Synthetic Gene

-20 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala  
 ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GCA  
 -10  
 -1 Tyr Ala  
 NsiI  
 1  
 +1 Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln Leu Ala  
 79 TTC CCA ACT ATA CCA CTA AGT CGA CTA TTC GAT AAC GCT ATG CTT CGG GCC CAT CGT CTT CAT CAG CTA GCC  
 20  
 NheI  
 30 Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro  
 40  
 142 TTT GAC ACC TAC CAG GAG TTT GAA GAG GCC TAT ATC CCC AAG GAA CAG AAG TAT TCA TTC CTG CAG AAC CCC  
 PstI  
 StuI  
 50 Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Lys Ser Asn  
 60  
 214 CAG ACC TCC CTC TGT TTC TCA GAA TCG ATT CCG ACA CCC TCC AAT CGC GAG GAA ACA CAA CAG AAA TCC AAC  
 NruI  
 70  
 80 Leu Glu Leu Leu Arg Ile Ser Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val  
 CTA GAG CTC CTC CGC ATA AGC TTG CTG CTC ATC CAG TCG TGG CTC GAG CCC GTG CAG TTC CTG AGG AGT GTC  
 90  
 MstII  
 100 Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Gly  
 110  
 358 TTC GCC AAC AGC CTG GTC TAC GGC GCC TCT GAT TCG AAC GTG TAC GAC CTG CTG AAG GAC CTA GAG GAA GGG  
 AsuII  
 BamHI  
 120  
 130 Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser  
 430 ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCG CGG ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC  
 SacII  
 BglII  
 140  
 150 Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys  
 AAG TTC GAC ACA AAC TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC GGG CTG CTC TAC TGC TTC AGG AAG  
 160  
 170 Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe AM\*  
 574 GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC GTG CAG TGC CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG  
 MstI  
 PvuII  
 180  
 190

FIG.-8

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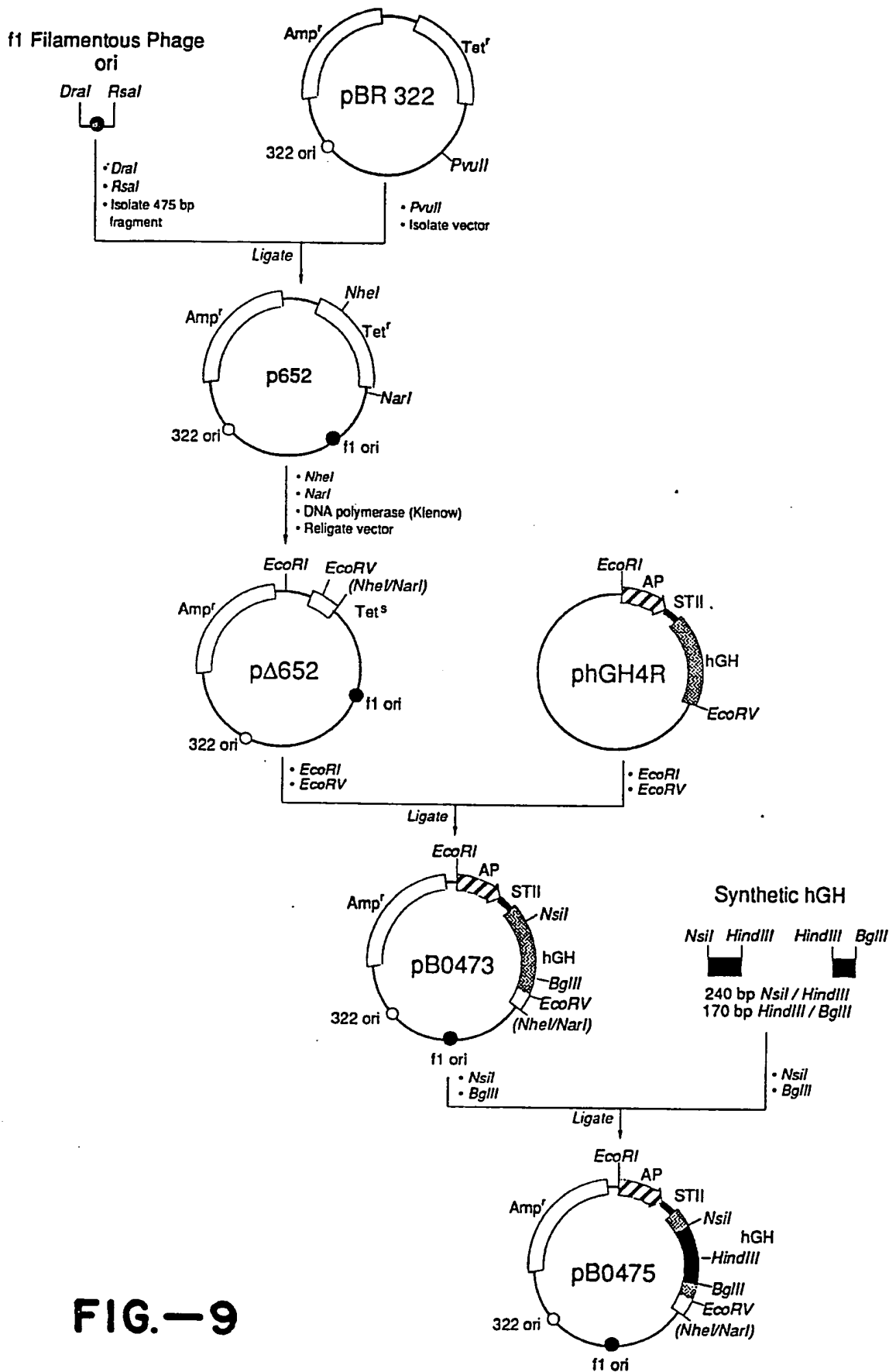


FIG.—9

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1 GATTCAACT TCTCCATACT TTGGATAAGG AATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTTGGA GATTATCGTC ACTGCAATGC  
 CTTAAGTGA AGAGGTATGA AACCTATTCC TTTATCTCTG TACTTTTAG AGTAACGACT CAACATAAA TTCGAAACCT CTAATAGCAG TGACGTTACG  
 101 TTCCGAATAT GGCCCAAAAT GACCAACAGC GGTGATGTA TCAGGTAGAG GGGCGGTGT ACGAGGTAAA GCCCGATGCC AGCATTCCTG ACGACGATAC  
 AAGGTTATA CCGGTTTATA CTGGTTGTG CCAACTAACT AGTCCATCTC CCCCAGGACA TGTCTCCATTT CCGGCTACGG TCCTAAGGAC TGCTGCTATG  
 201 CGTCTGCTG CCGCATACG TAAAGAACTT ATTGAAAGCT CCTCGTCACT AAAAAGTTAA TCTTTTCAAC AGCTGTCTATA AAGTTGTAC GGCCGAGACT  
 CCTGACGAC GCCTAATGC ATTTCTTCAA TAACTTCGTA GGAGCAGTCA TTTTTCATTT AGAAACTGT TCGACAGTAT TCAACACAGT CCGGCTCTGA  
 301 TATAGTCGCT TTGTTTTTAT TTTTAAATGT ATTTCTAACT AGTAGGCAAG TTCACGTAAA AAGGGTATCT AGAGGTTGAG GTGATTTT ATG AAA  
 ATACAGCGA AACAAAAATA AAAAAATTACA TAAACATTGA TCAATGCTTC AAGTGCATTT TTCCCATAGA TCTCCAACTC CACTAAAA TAC TTT  
 Met Lys  
 395 AAG AAT ATC GCA TCT CTT GCA TCT TCT TCT TCT ATT GCT ACA AAT GCC TAT GCA TTC CCA ACT ATA CCA CTA ddeI  
 TTC TTA TAG CGT AAA GAA GAA CGT AGA TAC AAG CAA AAA AGA TAA CGA TGT TTA CGG ATA CGT AAG GGT TGA TAT GGT GAT  
 -21 Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Phe Pro Thr Ile Pro Leu  
 476 AGT CGA CTA TTC GAT AAC GCT ATG CTT CGG GCC CAT CGT CTT CAT CAG CTA GCC TTT GAC ACC TAC CAG GAG TTT GAA GAG  
 TCA GCT GAT AAG CTA TTG CGA TAC GAA GCC CGG GTA GCA GAA GTA GTC GAT CGG AAA CTG TGG ATG CTC AAA CTC TTT CTC  
 7 Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu Glu

aluI hindIII  
 msei  
 ddeI  
 nlaIII  
 sau3AI  
 mboI[dam-]  
 hinPI  
 hhaI  
 bclI[dam-]  
 mnlI  
 haeII  
 rsaI  
 mnlI  
 bsmI  
 haeIII  
 xmaIII  
 eagI  
 eaeI  
 cfrI  
 bsmI  
 aluI  
 pvuII  
 msei  
 foki  
 sfaNI  
 speI  
 rsaI  
 msei  
 xbaI  
 mnlI  
 mnlI  
 hphI  
 bsmI  
 nsII  
 avaII  
 ddeI  
 sau96I[M.haeIII-]  
 haeIII  
 asuI  
 sau96I[M.haeIII-]  
 nlaIV  
 hgiJII  
 bspI286[M.haeIII-]  
 banII[M.haeIII-]  
 asuI  
 mboII  
 apaI  
 nheI[M.aluI-]  
 aluI  
 scrFI[dcn-]  
 ecorII  
 bstNI  
 mboII haeIII  
 earI haeI  
 GAA GAG  
 CTC CTC  
 Glu Glu

FIG.-10A

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**FIG. 10B**





**FIG. 10E**

**FIG. -10F**





[illegible]

**FIG. -10H**

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sau3AI fnu4HI  
 mboI[dam-] haeIII  
 dpnI eaeI  
 pvuI cfri  
 4241 CCGATCGTTG TCAGAAAGTAA GTTGGCCGCA GTGTATACAC TCAATGGTAT nlaIII fnu4HI foki  
 GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG AGTACCAATA CCGTCGTGAC GTATTAAGAG CATAATTC TCATGTTCAT nlaIII sfanI  
 TCTACGAAAA  
 hphI rsaI  
 bsrI scaI  
 4341 CTGTGACTGG TGACTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGCAACA CGGGATAATA bstUI[M.hhai-]  
 GAACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT CACATACGCC GCTGGCTCAA CGAGAACGG CCGCACTGT GCCCTATTAT CCGCGCGTGT  
 msel hgiAI  
 draI bspI286  
 4441 TAGCAGAACT TTAAGAAGTGC TCATCATTTG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT bspI  
 ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA AGCCCGCTT TTGAGAGTTC CTAGAATGC GCAACTCTA GGTCAAGCTA bspI  
 hgiAI sau3AI  
 bspI286 mboI[dam-] dpnI alwI  
 4541 CGTGCACCCA ACIGATCTTC AGCATCTTTT ACTTTCACCA CCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCG TTTTACGGG TTTTCCCT ATAGGGCGA  
 GCACGTGGGT TGACTAGAG TCGTAGAAA TGAAGTGT CGCAAGACC CACTCGTTT TGTCCTCCG TTTTCCCT TATCCCGT fnu4HI  
 hphI hphI  
 mboII[dam-] sau3AI  
 hgiAI bspI286 mboI[dam-] dpnI alwI xhoII bstYI taqI  
 4641 CACGGAATG TTGAATACTC ATACTCTTCC TTTTCAATA TTATTGAAGC ATTATCAGG GTTATTGTCT CATGAGCGGA TACATATTG AATGATTTA  
 GTGCCTTAC AACTTATGAG TATGAGAAGG AAAAGTTAT AATAACTTCG TAATAGTCC CAATAACAGA GTACTCCCT ATGTATAAC TTACATAAAT  
 mboII eaeI  
 mboII sspI  
 nlaIII bspH  
 bstAI

FIG.-101

## SI IRSTITI TE QUERT

**FIG. -10J**

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baII(GCCNNNNNGGC):  
 baII(AGATCT):  
 baII(GAATGC):  
 baII(CTCTC):  
 baII(1285(GDGGCHC):  
 baII(TC-IGA):  
 baII(ACCTGC):  
 baII(TCCGGA):  
 baII(CTGG):  
 baII(TTCGA):  
 baII(CCMGG):  
 baII(GCGG):  
 baII(CCA-NNNNNTGG):  
 baII(AGATCT):  
 baII(CCTNAGG):  
 baII(CCSGG):  
 baII(YGGCCR):  
 baII(ACCGAT):  
 baII(CTNAG):  
 baII(GATC):  
 baII(TTAA):  
 baII(CACNNNGTG):  
 baII(YGGCCR):  
 baII(CGGCCG):  
 baII(CTCTTC):  
 baII(CCTNAGG):  
 baII(CCTNNNNAGG):  
 baII(1091(RGGNCCY):  
 baII(GAATTC):  
 baII(CCMGG):  
 baII(GATATC):  
 baII(GCNGC):  
 baII(CGCG):  
 baII(GGATG):  
 baII(TGGCA):  
 baII(WGGCCW):  
 baII(RGGCCY):  
 baII(GGCC):  
 3989[M.haeIII-]  
 867  
 182 455 1390  
 295 977 2631 3942 4707  
 504[M.haeIII-] 677[M.aluI-] 719 1502 2408 2798 3296 4457 4542  
 3702 4710 4815  
 792  
 1701 2108 2568  
 706 860 1220 1547 1818 1842 2250 2729 2757 3385 3398 3515 3921 4039 4082 4346  
 4521  
 778  
 541 757 1140 1479 3009 3130 3143  
 211[M.haeI-] 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520[M.haeI-] 2540[M.haeI-]  
 2564[M.haeI-] 2582[M.haeI-] 2584[M.haeI-] 2687[M.haeI-] 3028 3609[M.haeI-] 3939  
 4432[M.haeI-] 4764[M.haeI-]  
 750  
 816 867 1704 2105 2571 3623 3634 3720 3732 4500 4517  
 733  
 1180 1295 1521 1849 2627 2662 3361 4057 4408  
 290 1481 4263  
 625  
 57 473 619 734 1618 1780 2792 3257 3666 3832 4372 4798  
 139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
 4243 4501 4518 4554  
 3739 3758 4450  
 2332  
 290 1481 4263  
 290  
 551 2860 4664  
 733  
 793  
 801 1475[dcM-] 1517 4850  
 1  
 541 757 1140 1479 3009 3130 3143  
 1195  
 204 207 697 849 940 1002 1017 1033 1236 1245 1324 1443 1446 1453 1467 1596 1599  
 1722 1803 2516 2538 2552 2621 2718 2771 2887 2905 2908 3026 3181 3324 3389 3392  
 3598 3926 4115 4265 4292 4387 4616  
 211 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520 2540 2564 2582 2584  
 2887 3028 3609 3939 4432 4764  
 238 703 1122 1143 1718 1807 1885 2046 2657 3855 4036 4323  
 987 1393 1491 4095  
 555 1481 2995 3006 3458  
 153 767 1242 1681 1764 2484 2492 2856 3226  
 291 505 556 1183 1298 1482 1986 2186 2328 2996 3007 3025 3459 3917 3997 4264  
 4851

FIG.—10K

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hgai(GACGC):  
 hgiAI(GAGCWC):  
 hgiCI(GGYRCC):  
 hgiJII(GGCCYC):  
 hhaI(GCGC):  
 hlnPI(GCGC):  
 hincII(GTYRAC):  
 hindII(GTYRAC):  
 71 691  
 E:ndIIII(AAGCIT):  
 h:infII(GAATC):  
 h:aiII(GCGG):  
 hphi(GGTGA):  
 mboII(GAAGA):  
 mpei(dam-)(GATC):  
 mnlI(CCIC):  
 mpei(TTAA):  
 mmpi(CCGG):  
 msti(TCCGCA):  
 mstiI(CCTNAGG):  
 mael(GCCGGC):  
 nari(GCCGCC):  
 nciI(CCSGG):  
 ndei(CATATG):  
 nhei(GCTAGC):  
 nlaIII(CATG):  
 nlaIV(GGNCCC):  
 nruI(TCCGCA):  
 nsiI(ATGCAT):  
 nspCix(RCATGY):  
 paer7I(CTCGAG):  
 pflMI(CCANNNNTGG):  
 pleI(GATC):  
 ppumi(RGGWCCY):  
 pstI(CTCGAG):  
 917 1277 1427 2041 2565 2688 3084 3662 4412  
 677[M.aluI-] 1502 2798 3296 4457 4542  
 767 1086 1129 1326 2374 3823  
 504 677 719 2408  
 112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
 2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
 112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
 2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
 477[M.taql-] 4414  
 477 4414  
 623[M.taql-] 628[M.taql-] 776[M.taql-] 1341[M.hphI-] 1562[M.hphI-] 2068 2264  
 2286 2882 2957 3353 3870  
 1171 1180 1295 1321 1522 1702 1849 2109 2439 2569 2628 2662 3189 3336 3362 3552  
 3956 3990 4057 4167 4409  
 380 1136 1344 1565 2346 2592 2601 3726 3953 4349 4575 4590  
 409 514 551 744 842 870(dam-) 1638 2465 2861 3632(dam-) 3723(dam-) 4478 4556(dam-)  
 4665 4861  
 139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
 4243 4501 4518 4554  
 148 163 241 372 378 554 606 610 639 650 682 736 771 809 835 1013 1125 1185 1265  
 1303 1330 1516 1830 1888 1944 2372 2579 2609 2871 3097 3154 3421 3821 3902 4032  
 4238 4849  
 69 257 324 1044 1066 1757 1979 2011 2125 2136 2148 2159 2176 2274 2545 2763  
 3688 3740 3745 3759 3812 4047 4086 4451 4823  
 1171 1180 1295 1321 1522 1702 1849 2109[M.bamHI-] 2439 2569[M.bamHI-] 2628 2662  
 3189 3336 3362 3552 3956 3990 4057 4167 4409  
 987 1393 1491 4095  
 733  
 1320 2438  
 767  
 1180 1295 1521 1849 2627 2662 3361 4057 4408  
 2804  
 523[M.aluI-] 1239  
 40 964 1288 1495 1629 1854 1918 1983 2618 2723 2983 3703 4194 4204 4282 4318  
 4711 4816  
 504 767 816 1086 1129 1291 1326 1361 1475 1518 1797 2105 2374 2395 2407 2571  
 3012 3051 3823 3917 3958 4169 4759  
 646  
 453  
 1853 2617 2982  
 716  
 14 1352 1401  
 2264 2286 2882 3353 3870  
 801 1475 1517  
 590 4116[M.HI-]

FIG.-10L

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PvuI(CGATCG):  
 PvuII(CAGCTG):  
 PstI(GTAC):  
 PciI(GAGCTC):  
 SacII(CCGCGG):  
 SalI(GTCGAC):  
 SmaI(GATC):  
 Sau96I(GGATCC):  
 SmaI(AGTACT):  
 SbfI(CCSGG):  
 SbfI(dcm-)(CCWGG):  
 SfiI(GCATC):  
 SnaBI(TACGTA):  
 SpeI(ACTAGT):  
 SspI(AATATT):  
 SstI(GAGCTC):  
 SbfI(AGGCGT):  
 SbfI(CCWGG):  
 SbfI(TCGA):  
 SbfI(CGGCG):  
 PstII(GACNNTGTC):  
 XbaI(TCIRGA):  
 XhoI(CTCGAG):  
 XhoII(RGATCY):  
 XmaIII(CGGCCG):  
 XmnI(GAANNNTTC):  
 not found:  
 AflII(CTTAAG), asp718(GGTACC), avrII(CCTAGG), bssHII(GCGCGC), bsteII(GGTNACC), espi(GCTNAGC), hpaI(GTTAAC),  
 kpnI(GGTACC), mluI(ACGGGT), ncoI(CCATGG), notI(GCGGCCG), rsrII(CGGWCCG), sfiI(GGCCNNNNNGGCC), smaI(CCCGGG),  
 sphiI(GCATGC), xmaI(CCCGGG)

4242  
 270 1018[M.HI-]  
 159 342 787 1174 2789 4354  
 677  
 854  
 477  
 139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
 4243 4501 4518 4554  
 504[M.haeIII-] 505[M.haeIII-] 802 1182[M.haeIII-] 1297[M.haeIII-] 1476[dcm-]  
 1518 1797 1986[M.haeIII-] 2328[M.haeIII-] 3917[M.haeIII-] 3996[M.haeIII-] 4013  
 4235 4851[M.haeIII-]  
 4353  
 1180 1295 1521 1849 2627 2662 3361 4057 4408  
 541 757 1140 1479 3009 3130 3143  
 175 237 416 990 1144 1214 1458 1710 1719 1806 1884 1947 2658 2774 2829 2850  
 3070 4122 4332 4562  
 217  
 338  
 2127 4677  
 677  
 535  
 567 1406  
 478 486 626[M.claI-] 717 779 894 975 1305 2370 3082 4526  
 211 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520 2540 2564 2582 2584  
 2687 3028 3609 3939 4432 4764  
 968 2726  
 368  
 716  
 816 867 1704 2105 2571 3623 3634 3720 3732 4500 4517  
 290  
 623 2068 4470

FIG.-10M

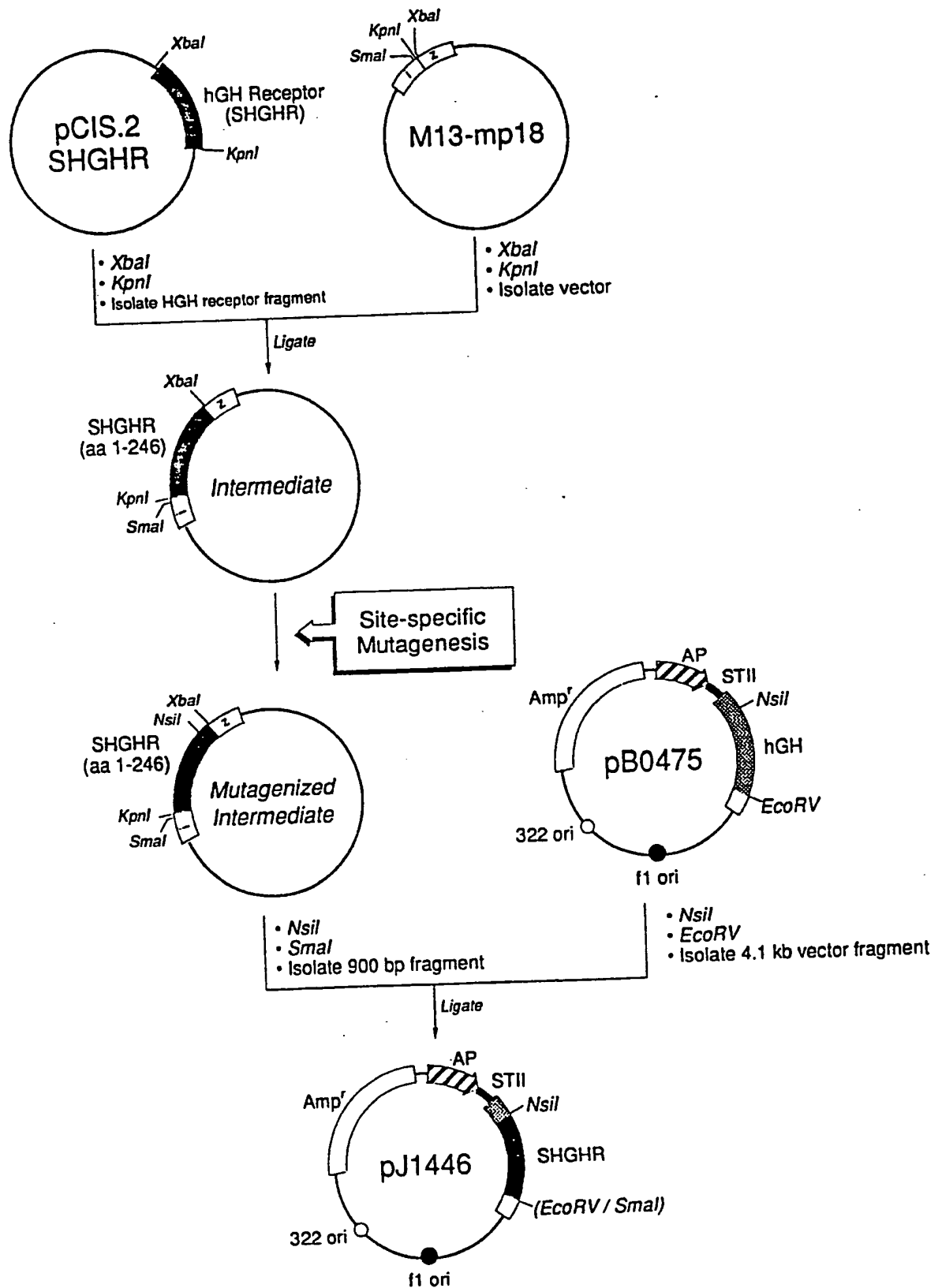


FIG.—II

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ecoRI      pflMI      nlaIII      ddeI      aluI      hindIII
1 GAATTCAACT TCTCCATACT TTGGATAAGG AATPACAGAC ATGAAAATC TCATTGCTGA GTTGTATTAT AAGCTTTGGA GATTATCGTC ACTGCAATGC
CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGCTCG TACTTTTAG AGTACGACT CAACAATAAA TTCGAAACCT CTAATAGCAG TGACGTTACG

sau3AI      mboI[dam-]      hinPI      hhaI      bclI[dam-]      mnlI      haeII      rsaI      mnlI      bsmI
101 TTGCGCAATAT GCGGCAAAAT GACCAACAGC GGTGATTGA TCAGGTAGAG GGGGCGCTGT ACGAGGTAAA GCCCGATGCC AGCATTCCCTG ACGACGATAC
AAGCGTTATA CCGGCTTTTA CTGGTTGTCG CCACTAACT AGTCCATCTC CCCCAGGACA TGCTCCATTT CGGCTACGG TCCTAAGGAC TGCTGCTATG

thai      fnu4HI      bvi      fnuDII      fnu4HI      bstUI[M.hhaI-]      bvi      hinPI      hhaI      snaBI      mnlI      foki      sfanI      msei
201 GGAGCTGCTG CCGGATTACG TAAGAAAGTT ATTGAAGCAT CCTCTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGTGCTATA AAGTTGTGAC GGCCGAGACT
CCTCGACGAC GCGCTAATGC ATTTCTTCAA TAACTTCGTA GGAGCAGTCA TTTTTCATTT AGAAAAGTTG TCGACAGTAT TTCAACAGTG CCGGCTCTGA

haeIII      xmaIII      eagI      eaeI      cfri      bsmal
301 TATAGTCGCT TTGTTTTTAT TTTTAAATGT ATTGTAACT AGTACGCAAG TTCACCTAAA AAGGGTATCT AGAGGTTGAG GTGATTTT
ATATCAGCGA AACAAAATA AAAAATTACA TAAACATTGA TCATCGGTTT AAGTGCATTT TTCCCATAGA TCTCCAATC CACTAAAA
Met Lys

mboII      sfanI
395 AAG AAT ATC GCA TTT CTT CTT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GCA TTT TCT GGA AGT GAG GCC
TTC TTA TAG CGT AAA GAA GAA CGT AGA TAC AAG CAA AAA AGA TAA CGA TGT TTA CGG ATA CGT AAA AGA CCT TCA CTC CGG
-21 Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Phe Ser Gly Ser Glu Ala

haeIII      stuI[dcn-]      haeI      scrFI[dcn-]      ecorII      bstNI      msei
476 ACA GCA GCT ATC CTT AGC AGA GCA CCC TGG AGT CTG CAA AGT GTT AAT CCA GGC CTA AAG ACA AAT TCT TCT AAG GAG CCT
TGT CGT CGA TAG GAA TCG TCT GGT ACC TCA GAC GAT TCA CAA TTA GGT CCG GAT TTC TGT TTA AGA AGA TTC CTC GGA
7 Thr Ala Ala Ile Leu Ser Arg Ala Pro Trp Ser Leu Gln Ser Val Asn Pro Gly Leu Lys Thr Asn Ser Ser Lys Glu Pro

aluI      fnu4HI      bvi      ddeI      hgiAI      ecorII      bstNI      hsp1286

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FIG.—12A

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draIII  
 hphI  
 557 AAA TTC ACC AAG TGC CGT TCA CCT GAG CGA GAG ACT TTT TCA TGC CAC TGG TGG ACA GAT GAG GTT CAT CAT GGT ACA AAG AAC  
 TTT AAG TGG TTC ACG GCA ACT GGA CTC GCT CTC TGA AAA AGT ACG GTG ACC TGT CTA CTC CAA GAT CCA GAT GGT TTC TTG  
 34 Lys Phe Thr Lys Cys Arg Ser Pro Glu Ser Phe Ser Cys His Trp Thr Asp Glu Val His His Gly Thr Lys Asn  
  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 ppuMI  
 ecoO109I  
 638 CTA CGA CCC ATA CAG CTG TTC TAT ACC AGA AGG AAC ACT CAA GAA TGG ACT CAA GAA TGG AAA GAA TGC CCT GAT TAT GTT  
 GAT CCT GGG TAT GTC GAC AAG ATA TGG TCT TCC TCG TGA GTT CTT ACC TGA GAT GAT GAT TTT CTT ACC TTT CTT GGA CTA ATA CAA  
 61 Leu Gly Pro Ile Gln Leu Phe Tyr Thr Arg Arg Asn Thr Gln Glu Trp Thr Thr Gln Glu Trp Lys Glu Cys Pro Asp Tyr Val  
  
 aluI  
 pvuII  
 719 TCT GCT GGG GAA AAC AGC TGT TAC TTT AAT TCA TCG TTT ACC TCC ATC TGG ATA CCT TAT TGT ATC AAG CTA ACT AGC AAT  
 AGA CGA CCC CTT TTG TCG ACA ATG AAA TTA AGT AGC AAA TGG AGG TAG ACC TAT GGA ATA ACA TAG TTC GAT TGA ICG TTA  
 88 Ser Ala Gly Glu Asn Ser Cys Tyr Phe Asn Ser Ser Phe Thr Ser Ile Trp Ile Pro Tyr Cys Ile Lys Leu Thr Ser Asn  
 these first 9 bases differ from 262 and 265  
  
 aluI  
 pvuII  
 800 GGT GCT GGG GAA AAC AGC TGT TAC TTT AAT TCA TCG TTT ACC TCC ATC TGG ATA CCT TAT TGT ATC AAG CTA ACT AGC AAT  
 CCA CCA TGT CAC CTA CTT TTC ACA AAG AGA CAA CTA CTT TAT CAC GTT GGT CTA GGT GGG TAA CGG GAG TTG ACC TGA AAT  
 115 Gly Thr Val Asp Glu Lys Cys Phe Ser Val Asp Glu Ile Val Gln Pro Asp Pro Pro Ile Ala Leu Asn Trp Thr Leu  
  
 rsal  
 foki  
 881 CTG AAC GTC AGT TTA ACT GGG ATT CAT GCA GAT ATC CAA GTG AGA TGG GAA GCA CCA CGC AAT GCA GAT ATT CAG AAA GGA  
 GAC TTG CAG TCA AAT TGA CCC TAA GTA CTA TAG GTT CAC TCT ACC CTT CGT GGT GCG TTA CGT CTA TAA GTC ATT CCT  
 142 Leu Asn Val Ser Leu Thr Gly Ile His Ala Asp Ile Gln Val Arg Trp Glu Ala Pro Arg Asn Ala Asp Ile Gln Lys Gly  
  
 bsrI  
 mser  
 962 TGG ATG GTT CTG GAG TAT GAA CTT CAA TAC AAA GAA GTA AAT GAA ACT AAA TGG AAA ATG ATG GAC CCT ATA TTG ACA ACA  
 ACC TAC CAA GAC CTC ATA CTT GAA GGT ATG TTT CTT CAT TTA CTT TGA TTT ACC TTT TAC TAC CTG GGA TAT AAC TGT TGT  
 169 Trp Met Val Leu Glu Tyr Glu Leu Gln Tyr Lys Lys Glu Thr Lys Trp Lys Met Met Asp Pro Ile Leu Thr Thr  
  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 foki  
 962 TGG ATG GTT CTG GAG TAT GAA CTT CAA TAC AAA GAA GTA AAT GAA ACT AAA TGG AAA ATG ATG GAC CCT ATA TTG ACA ACA  
 ACC TAC CAA GAC CTC ATA CTT GAA GGT ATG TTT CTT CAT TTA CTT TGA TTT ACC TTT TAC TAC CTG GGA TAT AAC TGT TGT  
 169 Trp Met Val Leu Glu Tyr Glu Leu Gln Tyr Lys Lys Glu Thr Lys Trp Lys Met Met Asp Pro Ile Leu Thr Thr

FIG.-12B

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sau3AI
mboI[dam-]
  dpnI
  alwI
xhoII
bstYI
bsrI rsal
1043 TCA GTT CCA GTG TAC TCA TTG AAA GTG GAT AAG GAA TAT GAA GTG CGT GTG AGA TCC AAA CAA CGA AAC TCT GGA AAT TAT
  AGT CAA GGI CAC AIG AGT AAC TTT CAC CTA TTC CTT ATA CTT CAC GCA CAC TCT AGG TTT GTT GCT TTG AGA CCT ATA ATA
196 Ser Val Pro Val Tyr Ser Leu Lys Val Asp Lys Glu Tyr Glu Val Arg Val Arg Ser Lys Gln Arg Asn Ser Gly Asn Tyr

          hgiAI          ddeI          nlaIII mboII          notI          haeIII
          bsp1286          mnlI          nspCIX mboII          fnu4HI          xmaIII
1124 GGC GAG TTC AGT GAG CTC TAT GTA ACA CTT CCT CAG ATG AGC CAA TTT ACA TGT GAA GAA GAT TTC TAC TAG CG
  CCG CTC AAG TCA CTC CAC GAG ATA CAT TGT GAA GCA GTC TAC TCG GTT AAA TGT ACA CTT CTT CTA AAG ATG ATC GC
223 Gly Glu Phe Ser Glu Val Leu Tyr Val Thr Leu Pro Gln Met Ser Gln Phe Thr Cys Glu Glu Asp Phe Tyr AM*

          mseI          aluI          bsmI
          hpaI          fnu4HI          TTTTCACTGC ATTCTAGTTG
          hindII          bbvI          AAAAGTGACG TAAGATCAAC
          hincII          CGTCCGCTTAA CTGTTTATT GCAGCTTATA ATGGTTACAA ATAAAGCAAT AGCATCACAA ATTTCACAAA TAAAGCATT TTTTCGTAAA
          thal          CGTCCGCAATT GAACAAATAA CGTCGAATAT TACCAATGTT TATTTCGTTA TCGTAGTGT TAAAGTGTT ATTTCGTAAA
          fnu4HI          sau3AI          mboI[dam-]          fnu4HI
          bstUI          dpnI          alwI          nheI hinPI
          fnu4HI          xhoII          nlaIV          fnu4HI hhai
          bsmI          bstYI          bamHI          bbvI haeII
          nlaIII alwI          sfaNI bsrI          CGTCTGCTA GCGCCGCCCT
          TCATGCTGG ATCCCATCGT CCATTCCGAC AGCATCGCA GTCACTATGG CAGTGATACC GCACGACGAT CGCGGCGGGA
          TACATAGAAAT AGTACAGACC TAGGGTAGCA GGTAAAGGCTG TCGTAGCGGT CAGTGATACC GCACGACGAT CGCGGCGGGA
1301 TGGTTTGTC CCAACTCATCA ATGTATCTTA TCATGCTGG ATCCCATCGT CCATTCCGAC AGCATCGCA GTCACTATGG CAGTGATACC GCACGACGAT CGCGGCGGGA
  ACCAAACAGG TTGAGTAGT TACATAGAAAT AGTACAGACC TAGGGTAGCA GGTAAAGGCTG TCGTAGCGGT CAGTGATACC GCACGACGAT CGCGGCGGGA

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FIG.-12C

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haeIII
sau96I[M.haeIII-]
scrFI
ncII
mspi
hpaiI
nlaIV
nlaIII
cauII
nlaIV
asui
taqI
mnli
nlaIV
hgiCI
mspi
hpaiI
bani
hphi
pflMI
1401 ATACCTTGTC TGCCTCCCCG CGTGGCTCG hgaI
TATGGAACAG ACGGAGGGGC GCAACGCAGC GCCACGTACC TCGGCCCGGT GACACGTGAC GGAGCTGGAC TACCTTCGG CCGCCGTGGA CGGATIGCCT AAGTGGTGAG
nlaIV
nlaIII
cauII
nlaIV
asui
taqI
mnli
nlaIV
hgiCI
mspi
hpaiI
bani
hphi
pflMI
1501 CAGGAATTGG AGCCAATCAA TTCTTGCGGA GAACTGTGAA TGCGCAAAACC AACCTTTGGC AGAACAATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC
GTCCTTAACC TCGGTTAGTT AGAAGCGCCT CTGACACTT ACSCGTTGG TTGGGAACCG TCTTGATATG GTAGCGCAGG CGGTAGAGGT CGTCGGCGTG
nlaIV
nlaIII
cauII
nlaIV
asui
taqI
mnli
nlaIV
hgiCI
mspi
hpaiI
bani
hphi
pflMI
1601 ATACCTTGTC TGCCTCCCCG CGTGGCTCG hgaI
TATGGAACAG ACGGAGGGGC GCAACGCAGC GCCACGTACC TCGGCCCGGT GACACGTGAC GGAGCTGGAC TACCTTCGG CCGCCGTGGA CGGATIGCCT AAGTGGTGAG
nlaIV
nlaIII
cauII
nlaIV
asui
taqI
mnli
nlaIV
hgiCI
mspi
hpaiI
bani
hphi
pflMI
1701 TACGAGAATG AATCACCAGT ACGCGAGCGA ACGTGAAGCG ACTGCTGTG CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTC
ATGCTCTTAC TTAGTGGCTA TCGGCTCGCT TGCACCTCGC TGACGACGAC GTTTTGCGA CGCTGGACTC GTTGTGTGAT TTACCAGAAG CCAAGAGCAC

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FIG.-12D

[illegible]

FIG. 12

2301	TTTTTGTATA TTTAAACAATT TTTAAACAATT	alul alul alul	msel msel msel	haeIII haeIII haeIII	ATAGGCGCGAA ATAGGCGCGCT ATAGGCGCGCT	TCCCTTTATA AGGGAATATT AGGGAATATT	ATCAAAAGAA TAGAGTATTA TAGAGTATTA	TAGAGTATTA TAGAGTATTA TAGAGTATTA	bsrI bsrI bsrI
2401	GTTTGGAA GTTTGGAA GTTTGGAA	pleI pleI pleI	msel msel msel	hinfI hinfI hinfI	GTGGACTTCA GTGGACTTCA GTGGACTTCA	CGTCAAAAGG CGTCAAAAGG CGTCAAAAGG	GCTATATCAGG GCTATATCAGG GCTATATCAGG	ACTACGTGAA ACTACGTGAA ACTACGTGAA	hphI hphI hphI
2501	TTTGGGTCG TTTGGGTCG TTTGGGTCG	hgiI hgiI hgiI	hgiI hgiI hgiI	hgiI hgiI hgiI	TTTGGGTCG TTTGGGTCG TTTGGGTCG	TTTGGGTCG TTTGGGTCG TTTGGGTCG	TTTGGGTCG TTTGGGTCG TTTGGGTCG	TTTGGGTCG TTTGGGTCG TTTGGGTCG	hgiI hgiI hgiI
2601	GAGTAAAGG GAGTAAAGG GAGTAAAGG	mbolI mbolI mbolI	mbolI mbolI mbolI	mbolI mbolI mbolI	GAGTAAAGG GAGTAAAGG GAGTAAAGG	GAGTAAAGG GAGTAAAGG GAGTAAAGG	GAGTAAAGG GAGTAAAGG GAGTAAAGG	GAGTAAAGG GAGTAAAGG GAGTAAAGG	mbolI mbolI mbolI
2701	CCGCTACAGG CCGCTACAGG CCGCTACAGG	ccgI ccgI ccgI	ccgI ccgI ccgI	ccgI ccgI ccgI	CCGCTACAGG CCGCTACAGG CCGCTACAGG	CCGCTACAGG CCGCTACAGG CCGCTACAGG	CCGCTACAGG CCGCTACAGG CCGCTACAGG	CCGCTACAGG CCGCTACAGG CCGCTACAGG	ccgI ccgI ccgI

**FIG.-12F**

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scrFI      hgaI      fnu4HI      bsrI      fnu4HI      ffaNI      ffaNI
ncII       thal      bbvI      tth1111   bsrI      ffaNI      ffaNI
mspi       fnuDII     hinPI      hhaI      nlaIII    ffaNI      ffaNI
hpaII      bstUI[M.hhaI-]  hhaI      hhaI      nlaIII    ffaNI      ffaNI
sfaNI      hhaI      hhaI      hhaI      nlaIII    ffaNI      ffaNI
foki cauII  hhaI      hhaI      hhaI      nlaIII    ffaNI      ffaNI
2801 AAGCGGATGC CGGGAGCAGA CAAGCCCGTC AGCGGGTGT GCGGGGTGTC GCGCCACAG CCCCGGTCG GTACTGGGC ATAGCGGAGT
TTCGCTACG GCCCTGCTCT GTTCGGGAC TCCCGGCGAG TCGCCACAA CGCCACAG CCGCGGTCG GTACTGGGC ATAGCGGAGT TATCGGCTCA

bsrI      sfaNI      ddeI      bspl286   hgaI      ffaNI      ffaNI
accI      msel      fnu4HI     rsal      apaLI    ndeI      ffaNI
2901 GTATCTGGC TTAACATGCG GCATCAGAG CAGATTGTAC TGAGAGTGCA CCATATGCGG TGTGAATATC CGCACAGATG CGTAAGGAGA AATATCCGCA
CATATGACCG AATTGATACG CCGTAGTCTC GTCTAACATG ACTCTACAGT GGTATACGCC ACACCTTATG CCGTGTCTAC GCATCTCTCT TTTATGGCGT

mboII     eari      hinPI      fnu4HI     ffaNI      ffaNI      ffaNI
hinPI     hhai      pleI      fnu4HI     ffaNI      ffaNI      ffaNI
hhaI      hhaI      hhaI      hhaI      ffaNI      ffaNI      ffaNI
haeII     hhaI      hhaI      hhaI      ffaNI      ffaNI      ffaNI
3001 TCAGGCGGTC TTCGCTTCC TCGCTACATG ACTCGCTGCG CTCGGTGTGT CGGCTGCGGC GACCGGTATC AGCTCACTCA AAGCGGGTAA TACGGTTATC
AGTCCGCGAG AAGCGGAGG AGCGAGTGAC TGAGCGACGC GAGCCAGCAA GCCGACGCGC CTCGCCATAG TCGAGTGAGT TTCGCCATT ATGCCAATAG

nlaIII     nlaIII     nlaIII     nlaIII     nlaIII     nlaIII     nlaIII
3101 CACAGAATCA GGGGATAACG CAGGAAGAA CATGTGAGCA AAGGCCAGC AAAGGCCAG GAACCGTAA AAGCGCGCGT TGCTGGCGTT TTTCCATAGG
GTGTCTTAGT CCCCTATTGC GTCCCTTCTT GTACACTCGT TTTCCGCTCG TTTCCGCTCG TTTCCGCTCG TTTCCGCTCG TTTCCGCTCG TTTCCGCTCG

scrFI[dcM-]  scrFI[dcM-]  scrFI[dcM-]  scrFI[dcM-]  scrFI[dcM-]  scrFI[dcM-]  scrFI[dcM-]
ecorII     ecorII     ecorII     ecorII     ecorII     ecorII     ecorII
bstNI      bstNI      bstNI      bstNI      bstNI      bstNI      bstNI
fnu4HI     fnu4HI     fnu4HI     fnu4HI     fnu4HI     fnu4HI     fnu4HI
haeIII     haeIII     haeIII     haeIII     haeIII     haeIII     haeIII
nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV
3201 CTCCGCCGCC CTGACGAGCA TCACAAAAT CGACGCTCAA GTCAAGGTG GCGAAACCG ACAGGACTAT AAGATACCA GCGGTTTCCC CCTGGAAGCT
GAGGCGGGG GACTGCTCGT AGTGTTTAA GCTGCGAGT CAGTCTCCAC CGCTTTGGGC TGCTCTGATA TTTCTATGCT CCGCAAGGG GGACCTTCGA

hgaI      taqI      sfaNI      hpaII     fnu4HI     hpaII     fnu4HI
3301 CCCTCGTGGC GACTGCTCGT AGTGTTTAA GCTGCGAGT CAGTCTCCAC CGCTTTGGGC TGCTCTGATA TTTCTATGCT CCGCAAGGG GGACCTTCGA
GGGAGCACGC GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAGAGG GAAGCCCTTC GCACCGGAA AGACTATCGA CACGCTGTAG
CATCATAGCT TCTCATAGCT CACGCTGTAG CACGCTGTAG CACGCTGTAG CACGCTGTAG CACGCTGTAG

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FIG.-12G

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3401 GTATCTCAGT TCGGTGTAGG TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT  
 CATAGAGTCA AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC GTGGGGGCA AGTGGGGCTG GCGACGGGA ATAGGCCATT GATAGCAGAA  
 hgiAI bsp1286 apalI aluI fnu4HI hpaII mspI hinPI hhaI fnu4HI hhaI  
 3501 GAGTCCACC CCGTAAGACA CGACTTATCG CCAGTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GACTTCTTGA  
 CTCAGGTTGG GCCATTCTGT CCGTAATAGC GGTGACCGTC GTCGGTGACC ATGTCTCTAA TCGTCTCGCT CCATACATCC GCCACGATGT CTCAGAGAACT  
 mspI hpaII scrFI nciI bspI bsvI bsri fnu4HI hpaII hhaI  
 3601 TGTGTTGCCC TAACACTAGG TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC  
 TACCCACCGG ATTGATGCCG ATGTGATCTT CCGTGCATTA ACCATAGACG CGAGACGACT TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG  
 haeIII haeI hinPI hhaI bsri fnu4HI hpaII mspI hpaII  
 3701 CCGCAACCAA ACCACCGCTG GTAGCGGTGG TTTTCTTTGTT TGCAAGCGAG AGATTACGCG CAGAAAAAAA GGATCTCAAG AGATCCCTTT GATCTTTTCT  
 GCCGTTTGT TGGTGGCGAC CATCGCCACC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCCG GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAAG  
 fnu4HI bsvI bstUI[M.hhaI-] aluI mboII[dam-] sau3AI mboII[dam-]  
 3801 ACGGGGTCTG ACGCTCAGTG GAACGAAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAAGGA TCTTCACCTA GATCCCTTTA AATTAAAAAT  
 TGGCCCGGAC TCGGAGTCAC CTGCTTTTG AGTGCAATTC CCTAAAAACCA GTACTCTAAT AGTTTTTCTT AGAAGTGGAT CTAGGAAAAAT TTAATTTTAA  
 hgaI ddeI mseI nlaIII bspHI aluI hpaI bsvI bstUI mboII[dam-] sau3AI mboII[dam-]  
 3901 CAAGTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTCA GGCACCTATC TCAGCGATCT GTCTATTTCG  
 CTTCAAAAAT TAGTTAGATT TCATATATAC TCATATATAC CAGACTGTCA ATGGTTAGCA ATTAGTCACT CCGTGGATAG AGTCGCTAGA CAGATAAAGC  
 mseI draI ahaIII hgiAI hgiAI hpaII mboII[dam-] sau3AI mboII[dam-]

FIG.-12H



**FIG. -121**

# INSTITUTE SHEET

hgiAI  
 bsp1286  
 1601 AAAGTGTCTC ATCATTTGAA AACGTTCTTC GGGGCGGAAA CTCTCAAGGA TCTTACCGCT GTTCAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAAC  
 TTTTCACGAG TAGTAACCTT TTGCAAGAAG CCCCCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG TCAAGCTACA TTGGGTGAGC ACGTGGGTG  
 mboII[dam-]  
 sau3AI  
 mboI[dam-]  
 dpnI sfaNI hphI  
 4701 TGATCTTCAG CAICTTTTAC TTTCACGAGC GTTTCGAGT hphI  
 ACTAGAAATC GTAGAAATG AAGAGGTGCG CAAAGACCCA CTCGTTTTG TCCTTCCGTT TTACGGCGTT TTTCCCTTA TTCCCGCTGT CGCTTACAA  
 mboII  
 earI sspI  
 4801 GAATACTCAT ACTCTTCCTT TTTCATATTT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTGAA TGTATTAGA AAAATAAACA  
 CTTATGAGTA TGAAGAAGAA AAGATATATA TAACCTCGTA AATAGTCCCA AATAGTCCCA ATAACAGAGT ACTCGCCTAT GTATAAACTT ACATAAATCT TTTTATTGT  
 hinPI  
 hhai  
 thal  
 fnuDII  
 nlaIV bstUI[M.hhai-]  
 4901 AATAGGGGTT CCGCGCACAT TTCCCGGAAA AGTGCACCT GACGTCTAAG AAACCATTTAT TATCATGACA TTAACCTATA AAAATAGGCG TATCACCAGG  
 TTTATCCCAA GCGCGGTGTA AAGSGGCTTT TCACGGTGA CTGCAGATTC TTTGGTAATA ATAGTACTGT AATTGGATAT TTTTATCCGC ATAGTGCTCC  
 mboII  
 5001 CCCTTTCGTC TTCAA  
 GGGAAAGCAG AAGTT  
 >>length: 5015  
 aatII(GACGTC): 4941  
 accII(GTNKAC): 2901  
 accII(TCCGGA): 1849 2256 2716  
 acvII(GRCGYC): 4559 4941  
 ahaII(GRCGYC): 4559[M.hpaiI-] 4941  
 ahaII(TTTAAA): 3887 3906 4598  
 ahaII(AAGCT): 72 203 271 481 651 734 786 1223 2184 2241 2314 2571 2771 2790 3071 3297 3387  
 3433 3690 4211 4311 4374

**FIG. -12J**

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fnu4HI(GC:GC): 204 207 479 1198 1201 1221 1384 1393 1472 1591 1594 1601 1615 1744 1747 1870  
 1951 2664 2686 2700 2769 2866 2919 3035 3053 3056 3174 3329 3472 3537 3540 3746  
 4074 4263 4413 4440 4535 4764  
 fnu2II(CGCG): 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2712 2730 2732 2835  
 3176 3757 4087 4580 4912  
 238 811 959 963 1866 1955 2033 2194 2805 4003 4184 4471  
 1541 1639 4243  
 471 526 1629 3143 3154 3606  
 haeI(TGCGCA): 153 1390 1829 1912 2632 2640 3004 3374  
 haeII(WGCCW): 291 472 527 1200 1446 1630 2134 2334 2476 3144 3155 3173 3607 4065 4145 4412  
 haeII(RGCGCY): 4999  
 haeII(GGCC): 1425 1575 2189 2713 2836 3232 3810 4560  
 hqa:(GACGC): 495 1139 1650 2946 3444 4605 4690  
 hgiAI(GAGCWC): 1474 2522 3971  
 hgiCI(GGYRCC): 2556  
 hgiII(GRGCCY): 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2711 2731  
 hhaI(GCGC): 2834 2864 3005 3038 3375 3475 3649 3758 4151 4244 4581 4913  
 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2711 2731  
 2834 2864 3005 3038 3308 3375 3475 3649 3758 4151 4244 4581 4913  
 hincII(GTYRAC): 1206 4562  
 hindII(GTYRAC): 1206 4562  
 hindIII(AAGCTT): 71  
 hinfI(GAATC): 505 685 901 1489[M.hphI-] 1710[M.hphI-] 2216 2412 2434 3030 3105 3501 4018  
 1206  
 hpaI(GTAAAC): 1443 1469 1670 1850 1997 2257 2587 2717 2776 2810 3337 3484 3510 3700 4104 4138  
 hpaII(CCGG): 4205 4315 4557  
 hphI(GGTGA): 380 561 575 1492 1713 2494 2740 2749 3874 4101 4497 4723 4738  
 mboII(GAAGA): 409 542 1181 1184 1786 2613 3009 3780[dam-] 3871[dam-] 4626 4704[dam-] 4813  
 5009  
 mboI[dam-](GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 mnlI(CCTC): 148 163 241 372 378 470 614 759 865 1136 1157 1413 1451 1478 1664 1978 2036  
 2092 2520 2727 2757 3019 3245 3302 3569 3969 4050 4180 4386 4997  
 maeI(TTA): 69 257 324 519 744 893 1207 1905 2127 2159 2273 2284 2296 2307 2324 2422 2693  
 2911 3836 3888 3893 3907 3960 4195 4234 4599 4971  
 mspI(CCGG): 1443 1469 1670 1850 1997 2257[M.bamHI-] 2587 2717[M.bamHI-] 2776 2810 3337 3484  
 3510 3700 4104 4138 4205 4315 4557  
 1541 1639 4243  
 mstI(TGCGCA): 1468 2586  
 naeI(GCCGGC): 1443 1669 1997 2775 2810 3509 4205 4556  
 nciI(CCSGG): 2952  
 ndeI(CATATG): 1387  
 nheI(GCTAGC): 1387

FIG.-12L

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nlaIII(CATG): 40 597 623 905 1176 1332 1436 1643 1777 2002 2066 2131 2766 2871 3131 3851 4342  
 nlaIV(GGNNCC): 4352 4430 4466 4859 4964  
 550 641 1024 1339 1439 1474 1509 1623 1666 1945 2253 2522 2543 2555 2719 3160  
 3199 3971 4065 4106 4317 4907  
 notI(GCGGCCGC): 1198  
 notII(ATGCCAT): 453  
 nspCIX(RCATGY): 1175 2001 2765 3130  
 nslMI(CCANNNNTGG): 14 1500 1549  
 nleI(GAGTC): 505 685 2412 2434 3030 3501 4018  
 ppuMI(RGGWCCY): 640 1623 1665  
 ptiI(CTGCCAG): 4264[M.HI-]  
 ptiI(CGATCG): 4390  
 pvuII(CAGCTG): 270 650 733  
 rsaI(GTAC): 159 342 627 804 1054 2937 4502  
 rsaIII(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 sau96I(GGNCC): 641 1024 1445[M.haeIII-] 1624[dcM-] 1666 1945 2134[M.haeIII-] 2476[M.haeIII-]  
 4065[M.haeIII-] 4144[M.haeIII-] 4161 4383 4999[M.haeIII-]  
 4501  
 scaI(AGTACT): 1443 1669 1997 2775 2810 3509 4205 4556  
 scrFI(CCSGG): 501 524 1627 3157 3278 3291  
 scrFI[dcM-](CCWGG): 175 237 416 1252 1362 1606 1858 1867 1954 2032 2095 2806 2922 2977 2998 3218  
 sfaKI(GCATC): 4270 4480 4710  
 217  
 snaBI(TACGTA): 338  
 speI(CTAGT): 338  
 sspI(AATATT): 2275 4825  
 stuI(AGGCGT): 526[dcM-]  
 styI(CCWGG): 637 1554  
 taqI(TCGA): 1453 2518 3230 4674  
 tthAI(CGCG): 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2688 2712 2730 2732 2835  
 3176 3757 4087 4580 4912  
 2874  
 tthIII(GACNNNGTC): 368  
 xbaI(TCTAGA): 850 1094 1339 1852 2253 2719 3771 3782 3868 3880 4648 4665  
 xhoII(RGATCY): 290 1199  
 xmaIII(CGGCCG): 2216 4618  
 xmnI(GAANNNTTC):  
 not found:

aflII(CTTAAG), apaI(GGGCCC), asp718(GGTACC), asuII(TTCGAA), bgII(AGATCT), bspMI(ACCTGC), bssHI(GCGCGC),  
 bstBI(TTCGAA), bstEI(GGTNACC), bstXI(CCANNNNTTGG), bsu36I(CCTNAGG), clai(ATCGAT), eco8I(CCTNAGG), ecoNI(CCTNNNNNAGG),  
 espI(CGTNAGC), kpnI(GGTACC), mluI(ACGGCT), mstII(CCTNAGG), nari(GGGGCC), ncoI(CCATGG), nruI(TCGGA), pae7I(CTCGAG),  
 rsfII(CGGWCCG), sacI(GAGCTC), sacII(CCGCGG), sali(GTCGAC), sfii(GGCCNNNNGGCC), smai(CCCGGG), sphi(GCATGC),  
 sstI(GAGCTC), xhoI(CTCGAG), xmaI(CCCGGG)

FIG.-12M

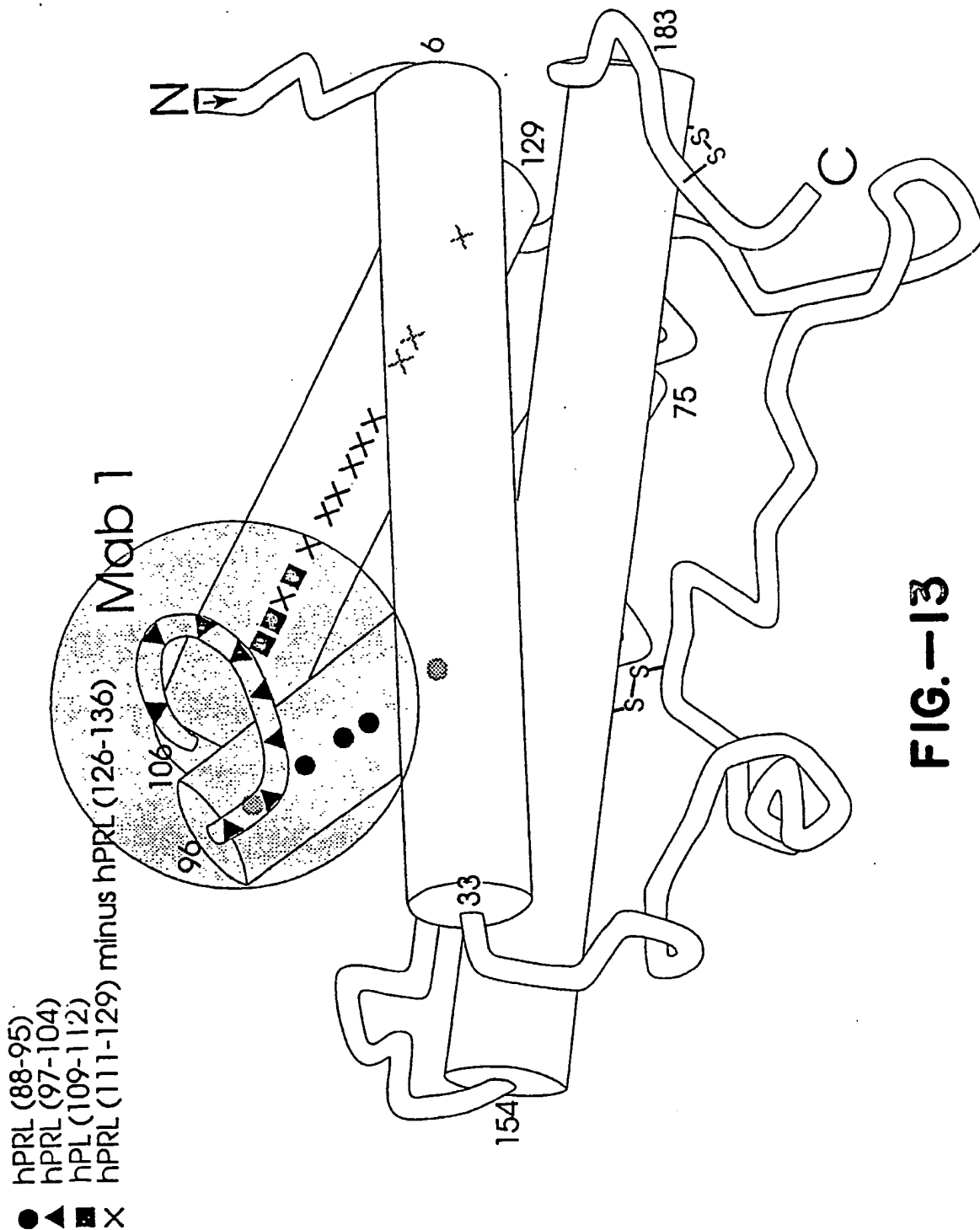


FIG.-13

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- pGH (11-33) minus hPRL (22-33)
- ▽ hPRL (12-19) minus hPL (12-25)
- ▲ hPRL (97-104)

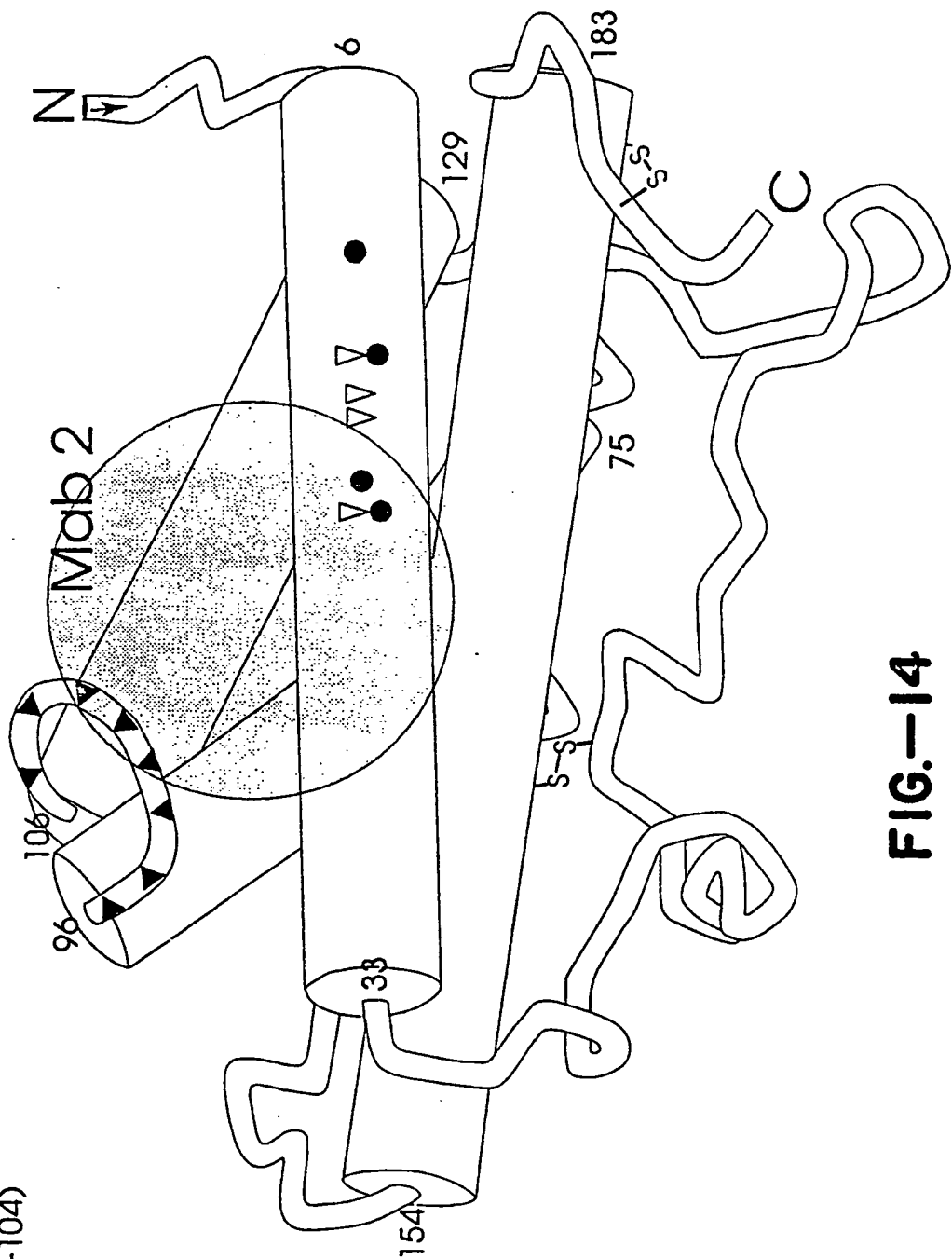


FIG.-14

- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

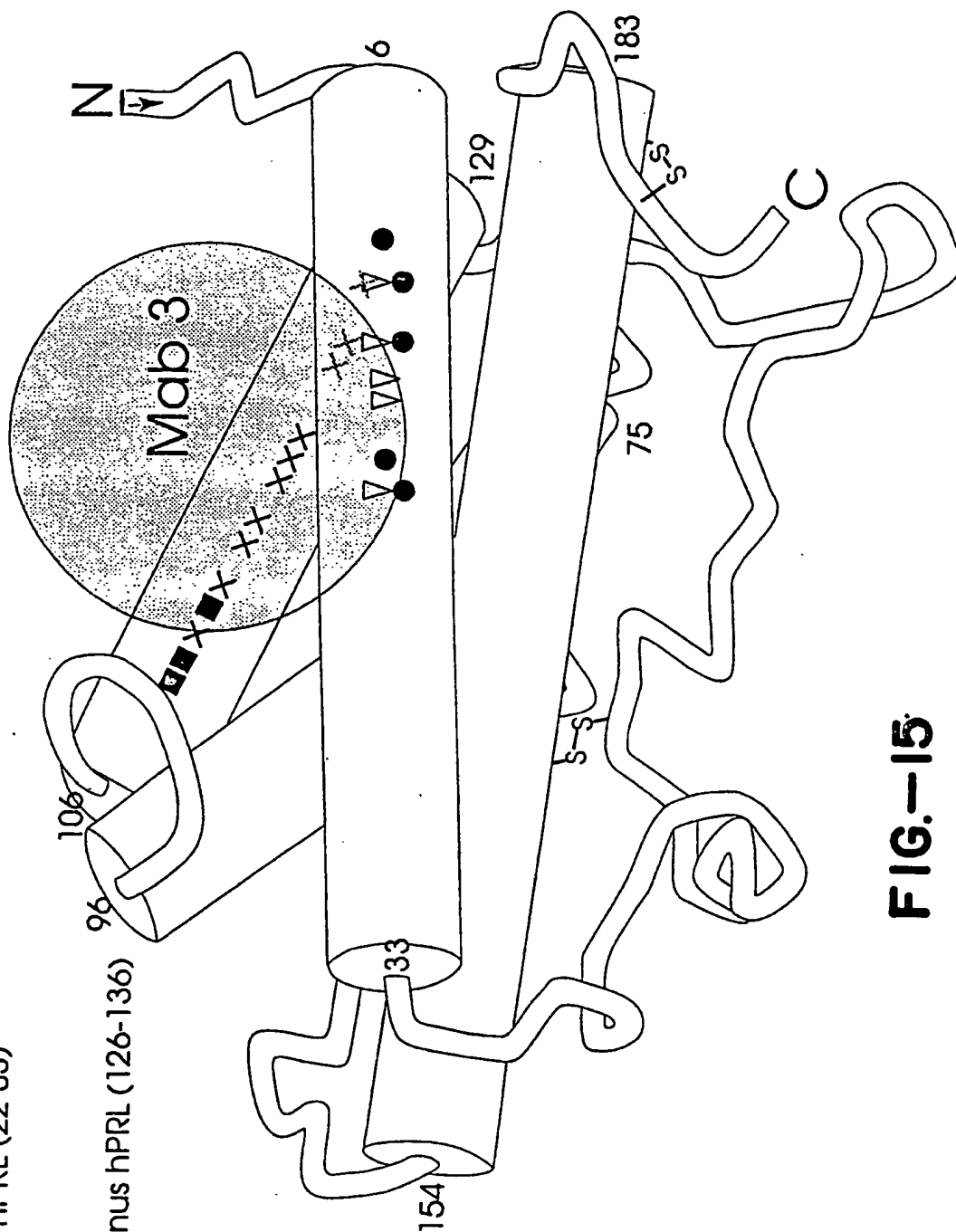


FIG.-15



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- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

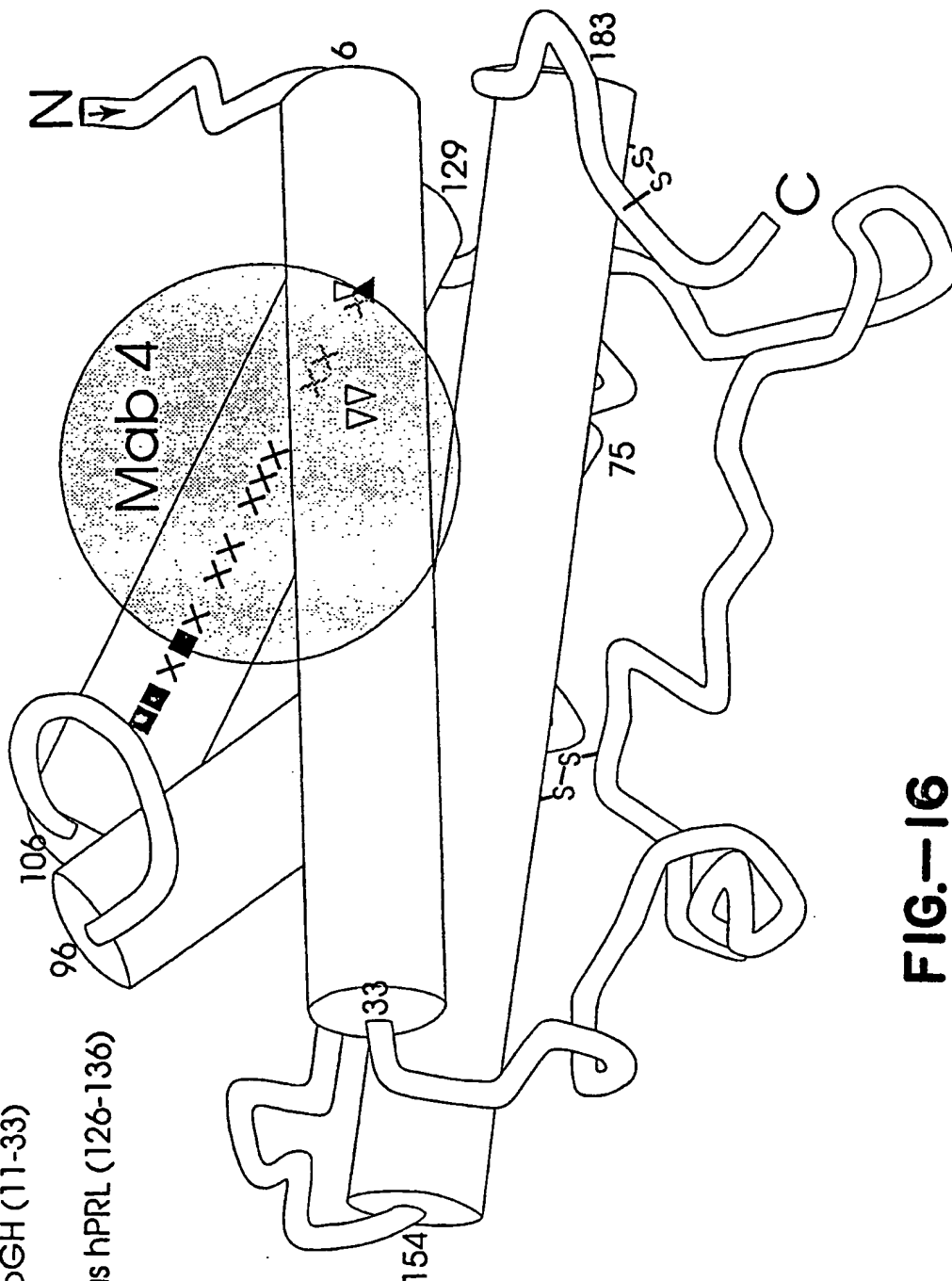


FIG.-16

**FIG. 17**

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- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)

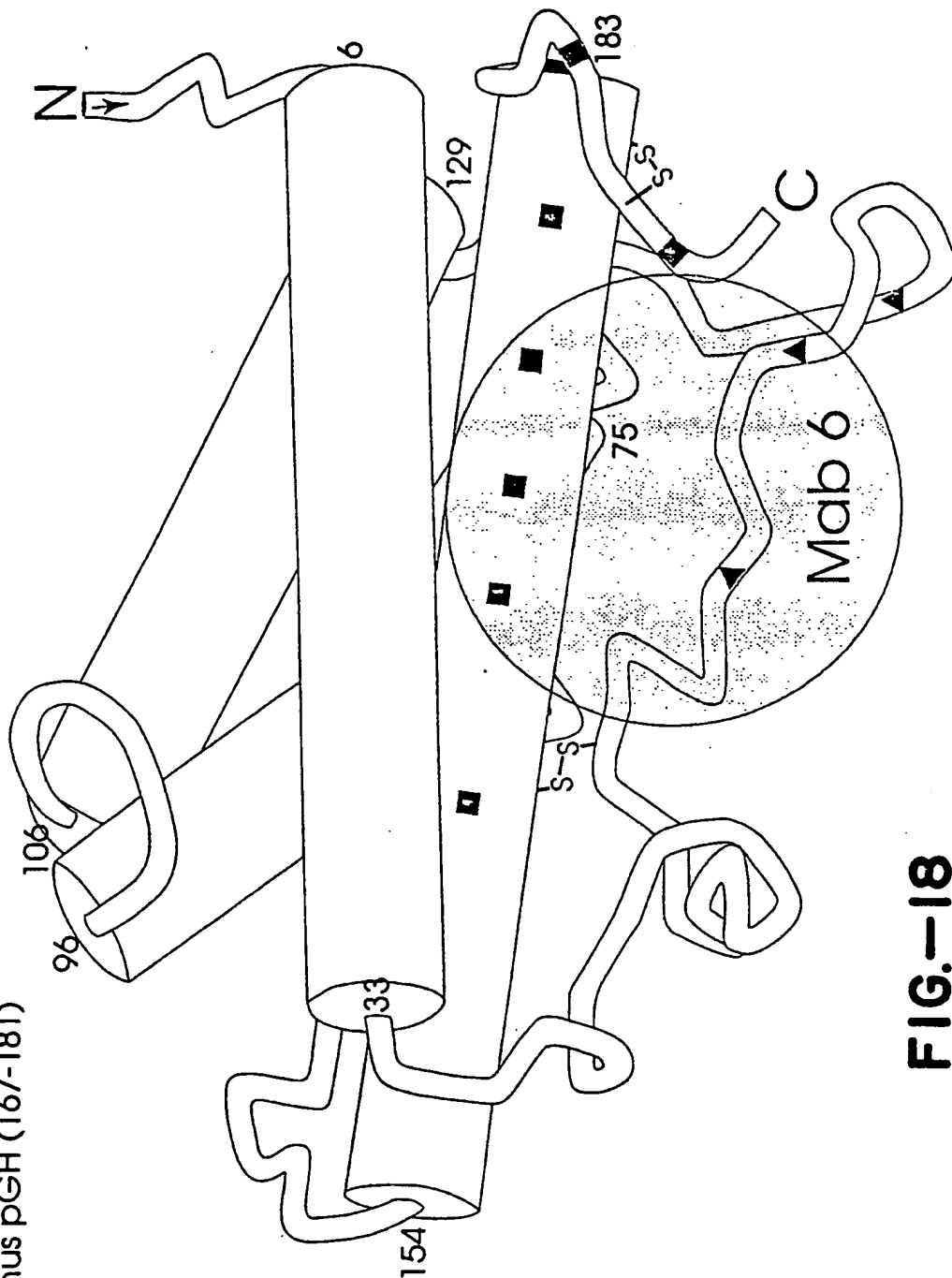
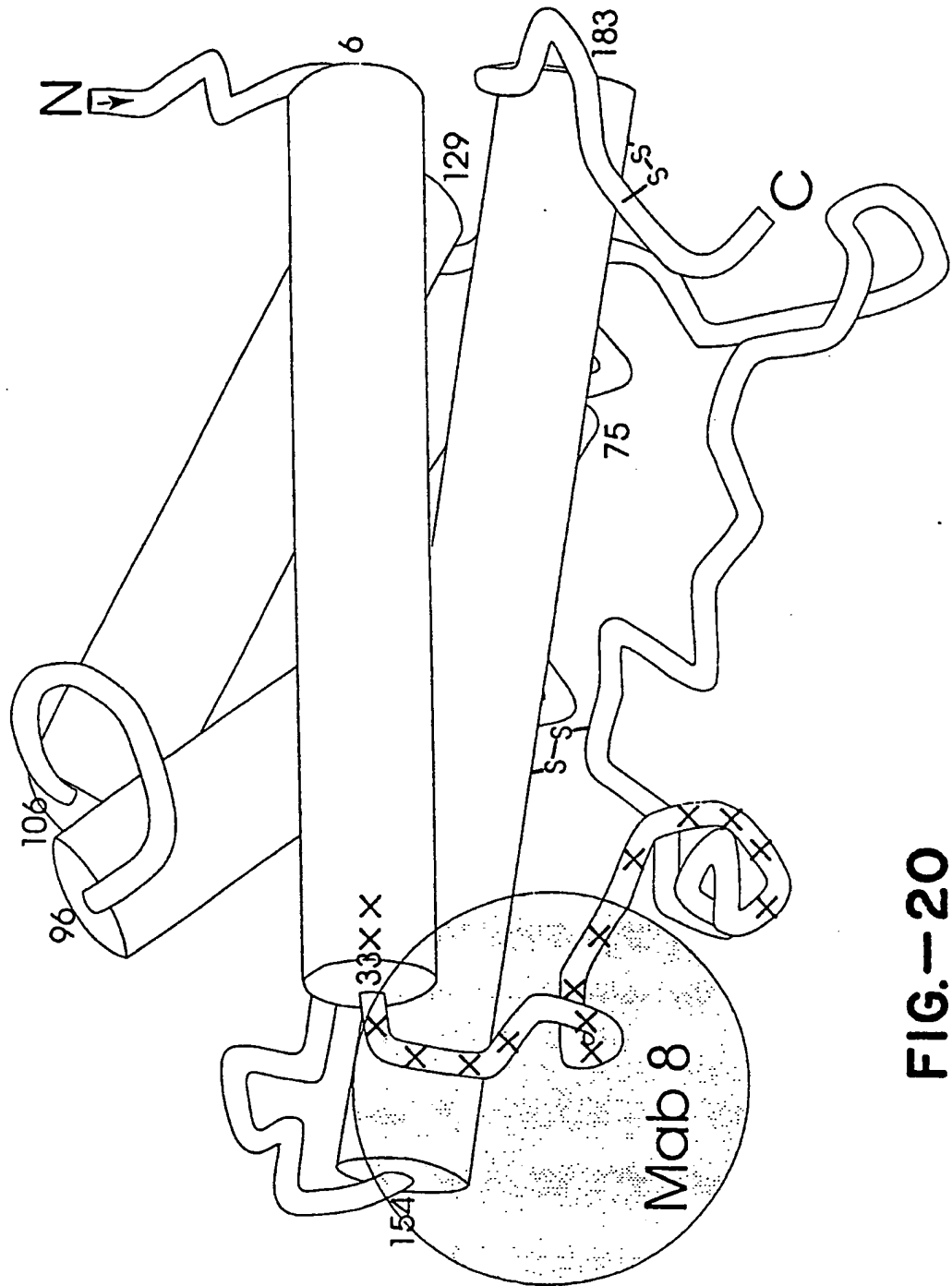


FIG.-18



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**FIG.-20**

X Deletion (32-46)

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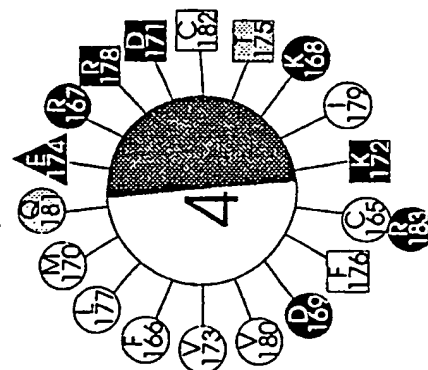
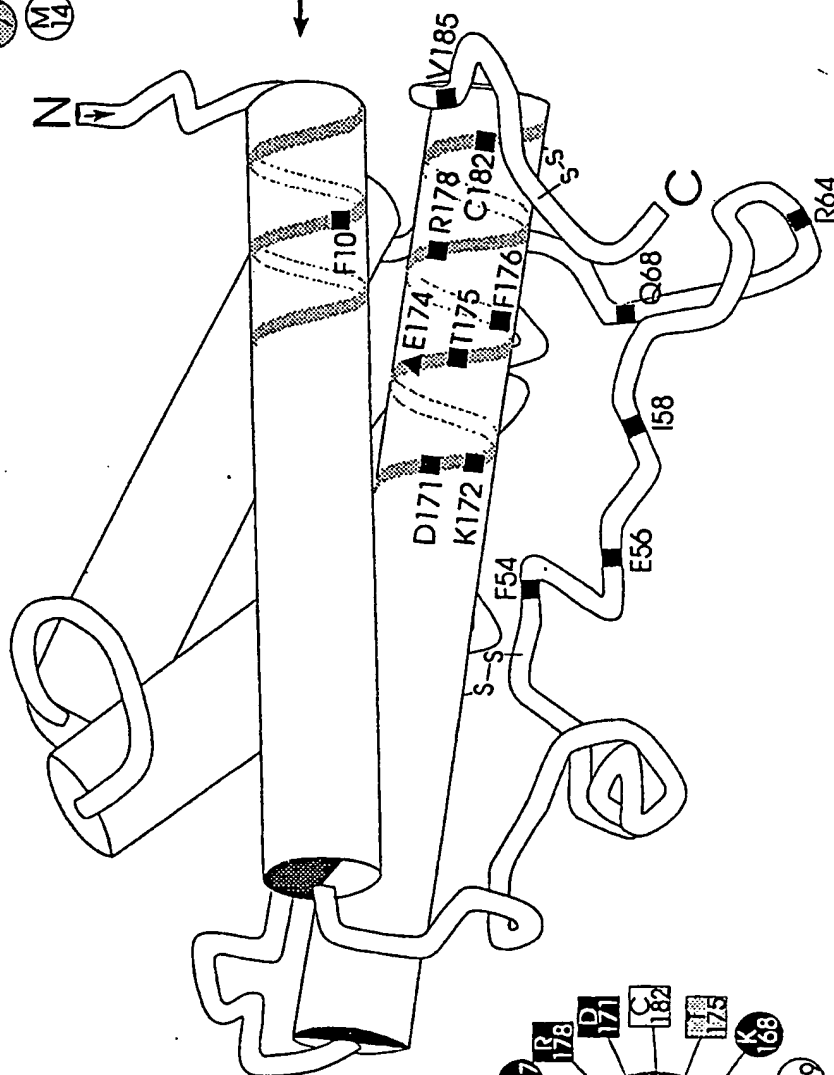
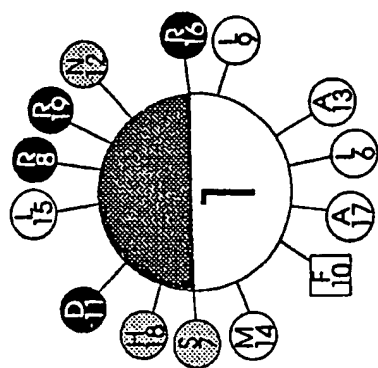


FIG.-21

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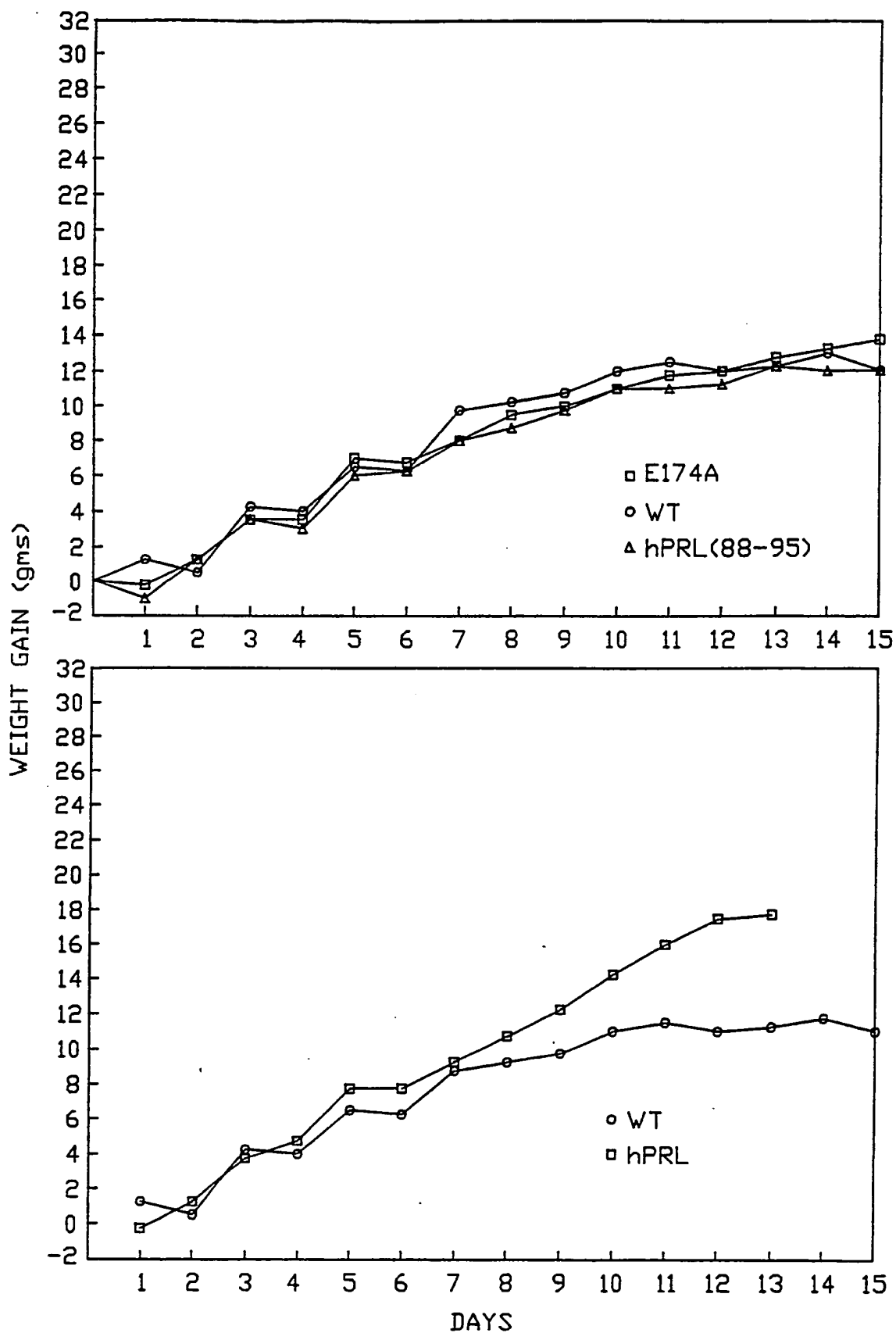


FIG.-22

SUBSTITUTE SHEET

## GH ANALOG BIO POTENCY IN RATS AFTER 8 DAYS OF TREATMENT

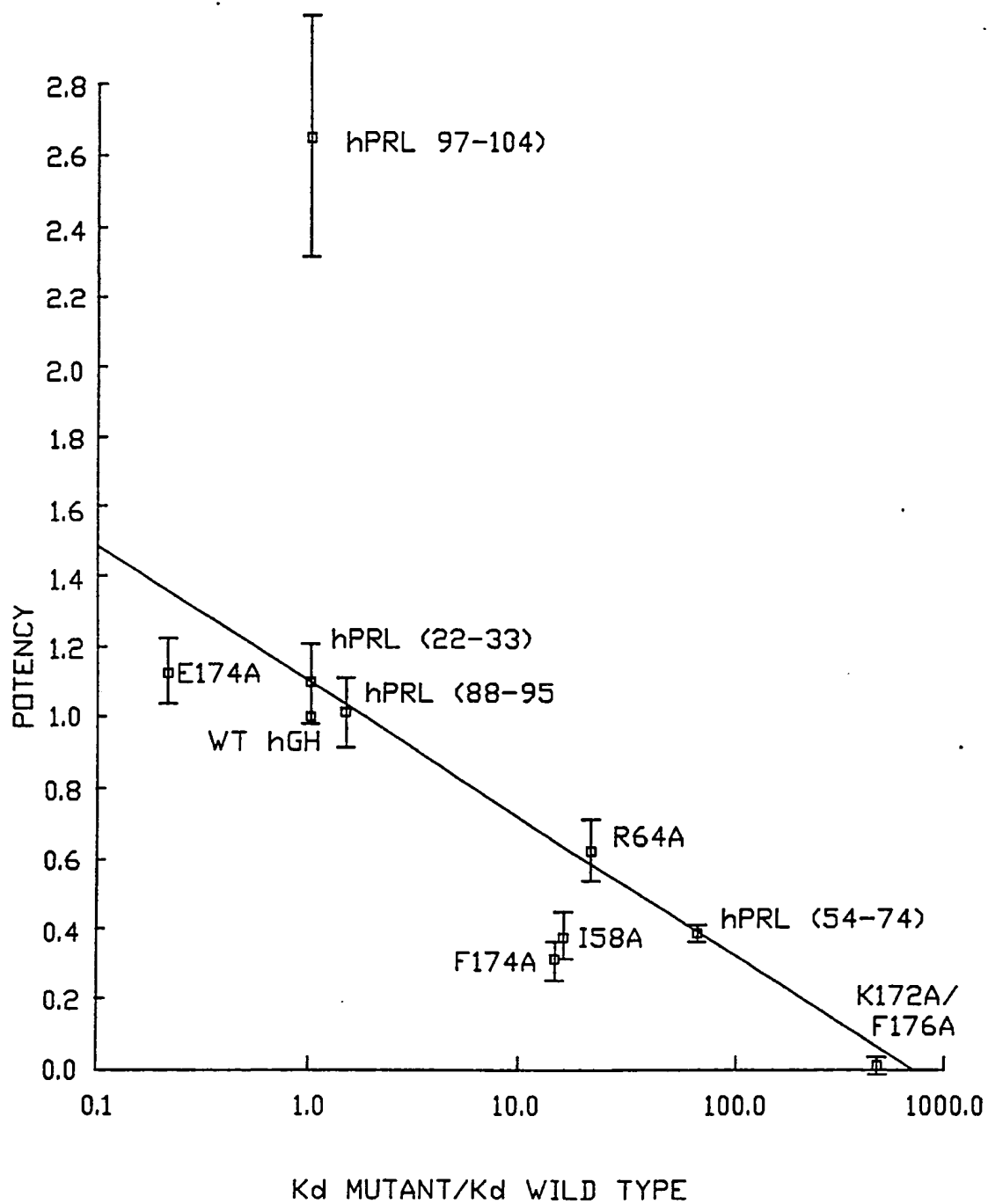


FIG.—23

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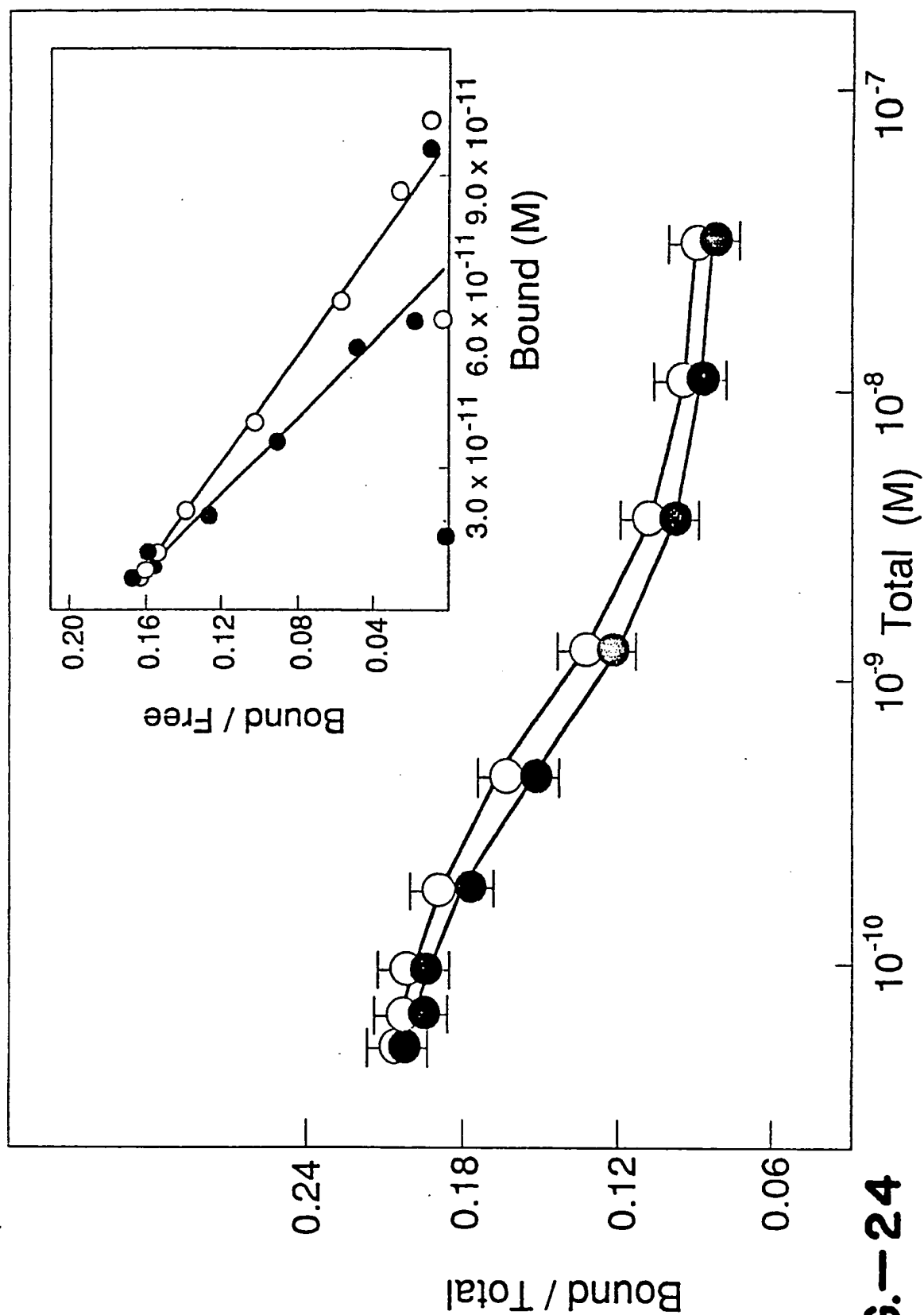


FIG.-24

SUBSTITUTE SHEET

## Binding Determinants for hGHR

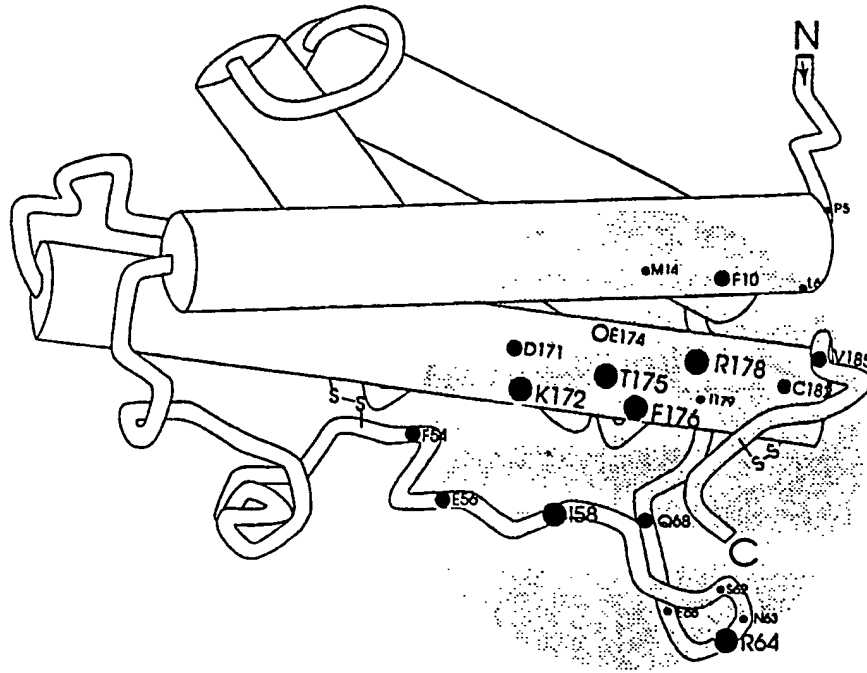


FIG.-25A

## Binding Determinants for hPRLr

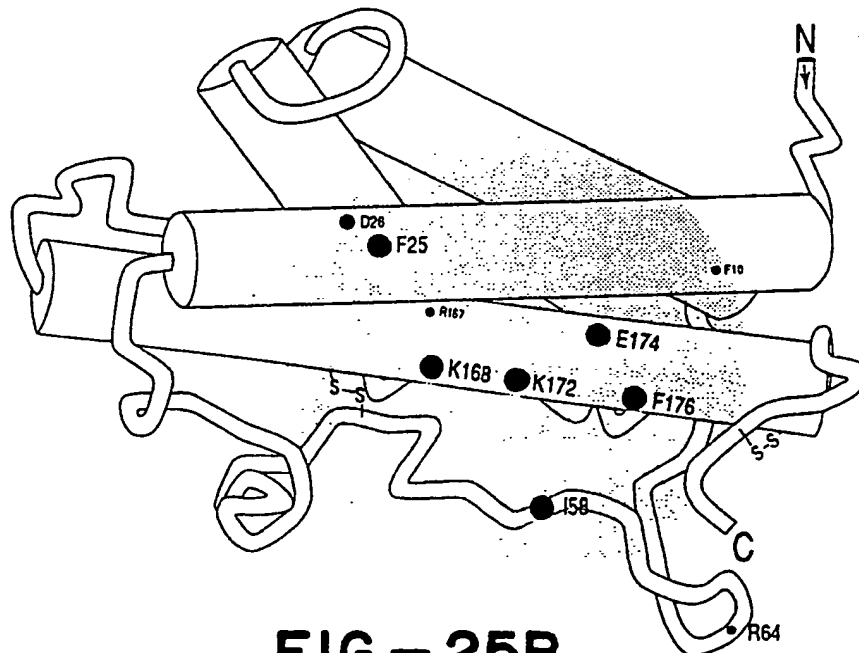


FIG.-25B

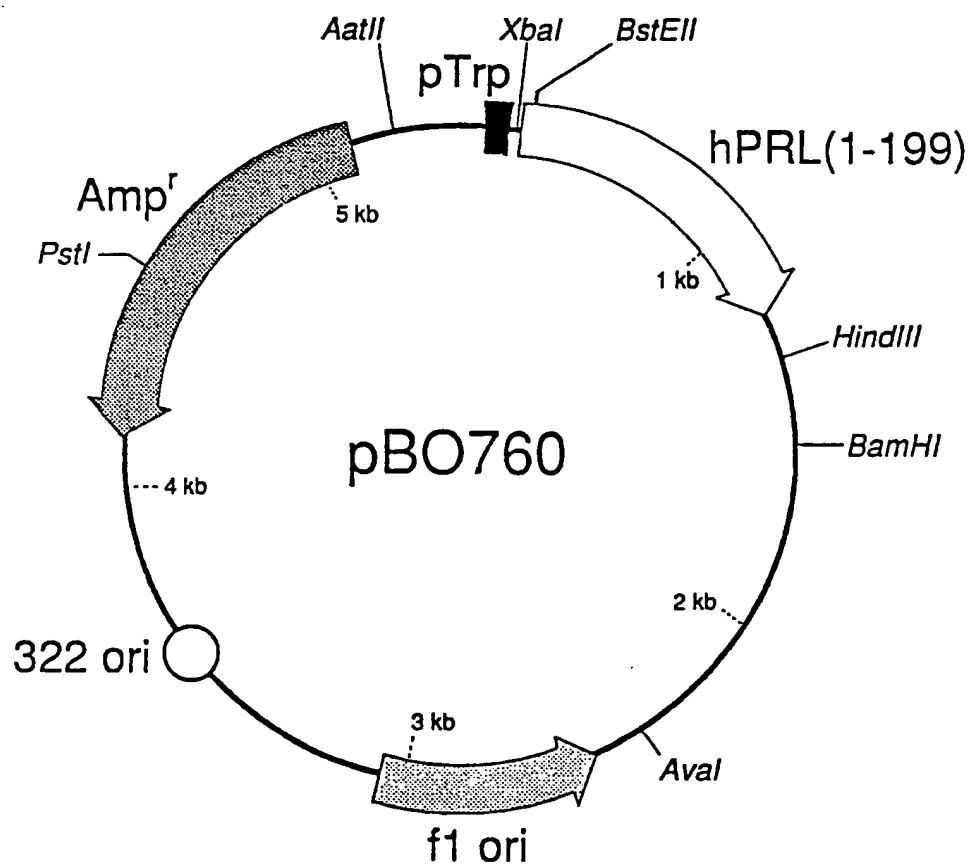
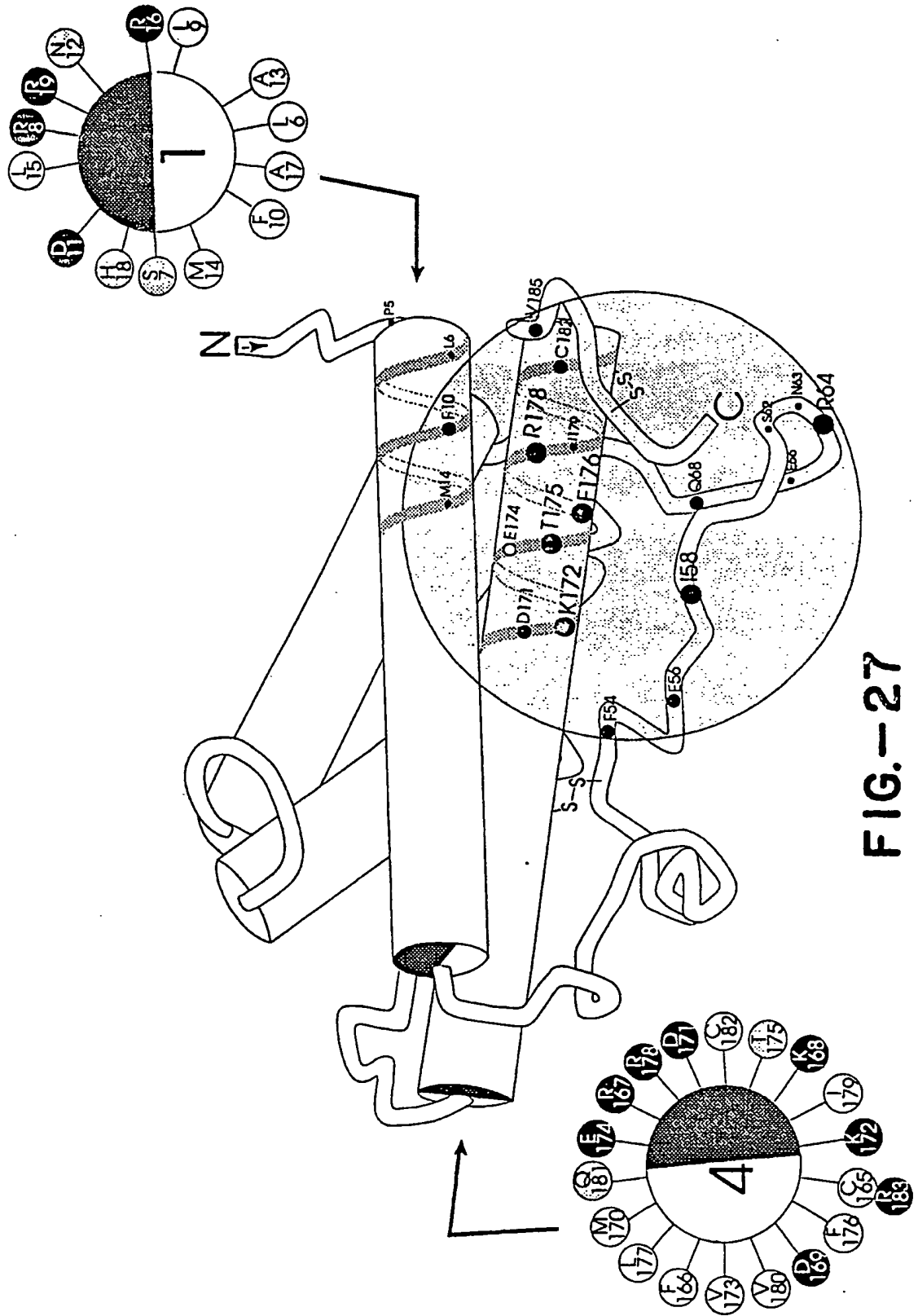
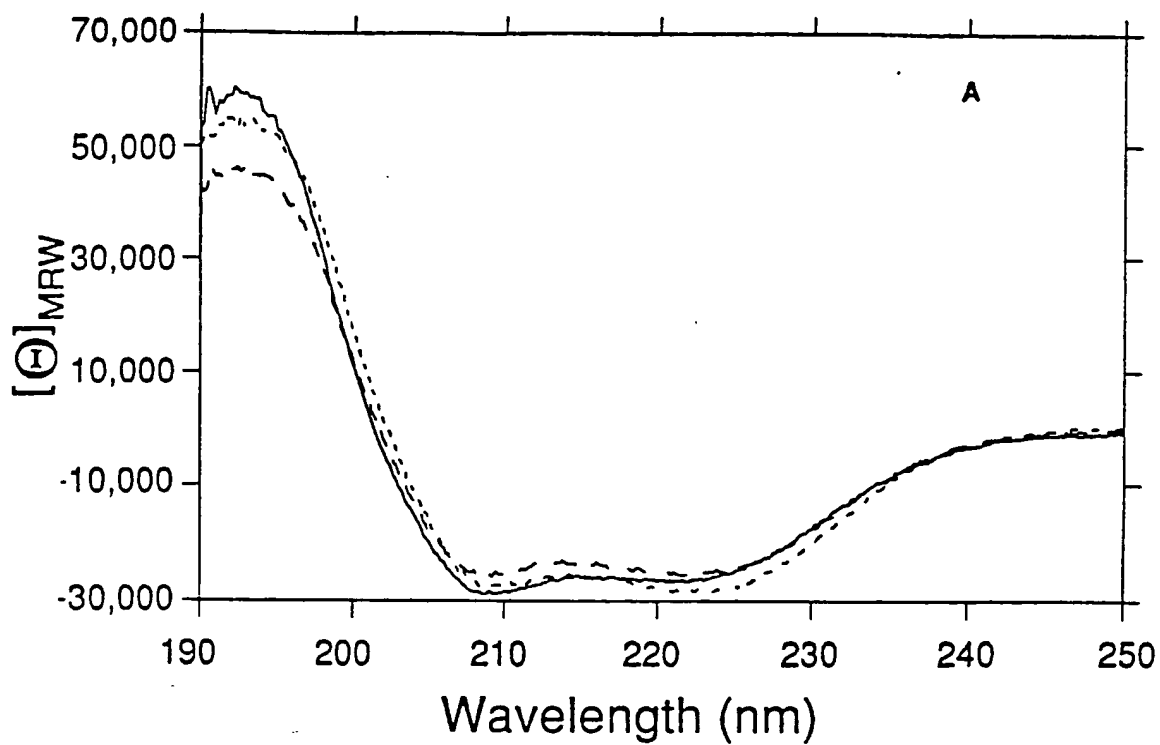
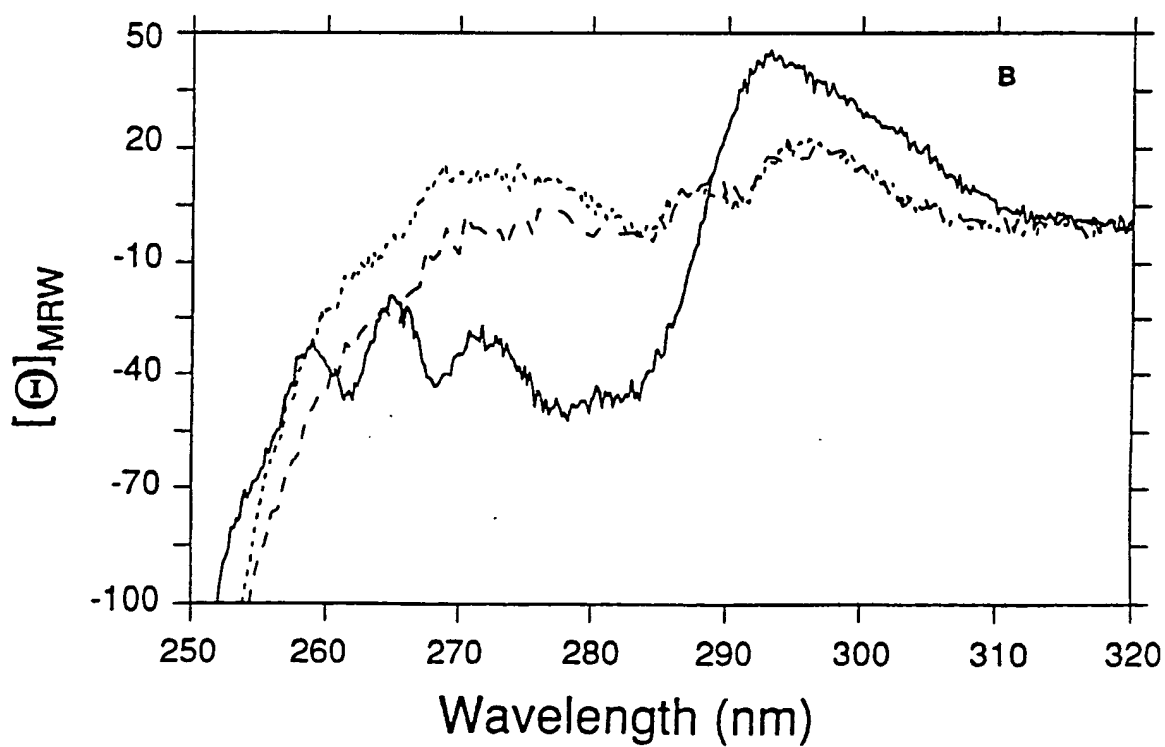


FIG.—26



**FIG.—28A****FIG.—28B**



## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04778

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): G01N 33/53, 31/00, 33/543, 33/567, 33/566 U.S. Cl: 435/7; 436/501, 504, 518, 548		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	435/7; 436/501, 504, 518, 548; 935/79, 81	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
CHEMICAL ABSTRACTS SERVICE ONLINE, BIOSIS PREVIEWS, AUTOMATED PATENT SYSTEM		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X <sup>5</sup> , P	Science, Volume 244, issued 1989, CUNNINGHAM, B.C., ET AL, "High Resolution Epitope Mapping of hGH-Receptor Interactions by Alanine-Scanning Mutagenesis", 1081-1085.	1-13, 16-31
X, P	Science, Volume 243, issued 1989, CUNNINGHAM, B.C., ET AL, "Receptor and Antibody Epitopes in Human Growth Hormone Identified by Homolog-Scanning Mutagenesis", 1330-1336.	1-13, 16-31
A	Biochem. Biophys. Res. Commun., Volume 135, issued 1986, SOUROUTON, M.C., ET AL, "Localization of a Highly Immunogenic Region on the Acetylcholine Receptor Alpha-Subunit", 82-89.	1-13, 16-31
<p><sup>10</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
02 February 1990	05 MAR 1990	
International Searching Authority	Signature of Authorized Officer	
ISA/US	KAREN I. KRUPEN	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Endocrinol., Volume 121, issued 1987, WERTHER ET AL, "Localization and Characterization of Insulin Receptors in Rat Brain and Pituitary Gland Using In-Vitro Autoradiography and Computerized Densitometry, 1562-1570.	1-13,16-31
A	Endocrinology, Volume 107, issued 1980 MILLS, T.B. ET AL, "Fragments of human growth hormone produced by digestion with thrombin: chemistry and biological properties", 391-399 (See Abstract, 143544)	1-13,16-31
A	Chemical Abstracts, Volume 108, no. 11, issued 1988, (Columbus, Ohio, U.S.A) B. C. Cunningham, "Improvement in the alkaline stability of subtilisin using an efficient random mutagenesis and screening procedure", Abstract.	1-13,16-31



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . . . because they relate to subject matter<sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . . . because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>13</sup>, specifically:

3. ☐ Claim numbers . . . . . because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

(See Attachment).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-13 and 16-31

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO PCT/ISA/210

Part IV. Before #1, Observations

- I. Claims 1-13 and 16-31 are drawn to a method for identifying unknown active domains in the amino acid sequence of polypeptides classified in class 436, subclass 501.
- II. Claims 14, 15 and 32-64 are drawn to a method of forming a growth hormone variant and the growth hormone variants produced classified in class 530, subclass 350.
- III. Claims 65-79 are drawn to human prolactin hormone variants classified in class 530, subclass 399.
- IV. Claims 80-83 are drawn to human placental lactogen variants classified in class 530, subclass 399.
- V. Claims 84-86 are drawn to DNA sequences and expression vectors and hosts classified in class 536, subclass 27.